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Cevitamic Acid Excretion in Pneumonias and Some Other Pathological Conditions.

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Previous studies by Harde,[1] and Harde and Philippe,[2] Harde and Benjamin,[3] Harde and Greenwald,[4] have shown a lowering of vitamin C content of the tissues of laboratory animals in many infections and intoxications. King[5] and his associates noted a similar

* P represents a preliminary, C a complete manuscript.

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1 Harde, E., *C. R. de l'acad. des Sc.*, 1934, **199**, 618.

2 Harde, E., and Philippe, *C. R. de l'acad. des Sc.*, 1934, **199**, 738.

3 Harde, E., and Benjamin, H. R., Proc. Soc. Exp. Biol. and Med., 1935, **32**, 651.

4 Greenwald, C., Harde, E., Proc. Soc. Exp. Biol. and Med., 1935, **32**, 1157.

5 Yavorsky, M., Almaden, P., and King, C. G., *J. Biol. Chem.*, 1934, **106**, 525.

fact in studies of human autopsies, and later in work on guinea pigs.[6]

These observations suggested to us that vitamin C in infectious diseases unrelated to scurvy, such as pneumonia, may be lowered. The examinations of urines for vitamin C from pneumonia and other pathological conditions permitted verification of this hypothesis.

A reducing substance in the urine of normal individuals, probably in large part cevitamic acid, has been studied by Hess and Benjamin[7] and by Birch, Harris and Ray.[8] The latter authors found a relation between vitamin C content of the diet and the urinary excretion of the reducing substance. On a balanced diet, normal adults excrete

TABLE I.
Pneumonia Cases.

Case No.	Diagnosis	Date	Mg. cevitamic acid excretion per cc. per diem	Mg. cevitamic acid excretion per diem	Dosage Mg. Cevitamic acid (Merck)	Remarks
1	Type II	2/18	.015	5.05		Peak not
	Serum	2/19	.006	12.75	200 mg.	reached until
		2/20	.010	13.42	100 mg.	8 days after
		2/21	.009	6.90	200 mg.	dosing.
		2/22	.007	16.14	400 mg.	Recovered.
		2/23	.012	10.58	200 mg.	
		2/24	—	—	400 mg.	
		2/25	.048	59.35	200 mg. 8 oz. orange ju.	
2	Type VIII	2/25	.012	1.63	400 mg.	No saturation
	Serum	2/26	.010	2.31	6 oz. or. ju.	after 10 days
		2/27	.012	4.49	400 mg.	dosing.
		2/28	.013	4.53		Recovered.
		—	—	—	—	
		—	—	—	—	
		—	—	—	—	
		3/6	.007	4.59	400 mg.	
3	Type I	2/28	.00	0.00	2×1-gm.	Massive dosing
	B. Friedlander A	3/1	.00	0.00	3×1-gm.	did not cause
		3/2	.017	13.66	None	immediate
	Serum	3/4	—	—	,,	saturation.
		3/5	.110	90.00	,,	Died.
		3/6	.110	68.95	,,	
4	Type VII			*		Recovered
	Serum	3/12	.016	4.80	1-gm.	
		3/13	.017	6.12	,,	
		3/14	.033	15.17		

[6] Bessey, O. A., and King, C. G., *J. Nutrition*, in press.

[7] Hess, A. F., and Benjamin, H. R., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 855.

[8] Birch, T. W., Harris, and Ray, *Biochem. J.*, 1933, **27**, 590.

TABLE I. (Continued).

Case No.	Diagnosis	Date	Mg. cevitamic acid excretion per cc. per diem	Mg. cevitamic acid excretion per diem	Dosage Mg. Cevitamic acid (Merck)	Remarks
5	Type V Serum	3/7	.018	8.55	No dosing	Recovered.
		3/8	.041	13.65		
		3/14	.012	7.60		
		3/15	.010	3.55		
6	Type VIII Serum	3/11	.018	12.85	2 500-mg.	Recovered.
		3/12	.028	36.70	2 500 "	
		3/13	.020	24.00	2 500 "	
		3/14	.039	33.03		
7	Type III Bactere-	2/20	.003	1.98	16 oz.	No saturation.
	mia Serum	2/21	.003	2.10	orange juice	Recovered.
		2/22	.010	1.75	daily	
		2/23	—	—		
		—	—	—		
		—	—	—		
		3/1	.008	2.40		
8	Type III Serum	2/24	—	—	400 mg.	Died
		2/25	.018	21.55	400 "	
		2/26	.021	17.85		
9	Type V Serum	5/1	.019	4.28	1 gm. 4 doses	Recoverd.
		5/2	.000	0.00		
		5/3	.000	0.00		
		—	—	—		
		—	—	—		
		5/7	.009	2.25		
		5/8	.005	3.00		
10	Type I Serum	4/24	.007	1.05	1 gm. 3 doses	No excretion.
		4/25	.000	0.00	1 " 3 "	Recovered.
		4/26	.000	0.00	1 " 3 "	
		4/27	.000	0.00		
11	Type I Non-	2/18	.022	7.46	3 100-mg.	Recovered.
	Serum	2/19	.009	3.36	doses	
		2/20	.008	1.12		
		2/21	.000	0.00		
12	Type VIII Non-	3/29	.008	2.64	250 mg.	Following peak patient continued to overflow with low dosing, then dropped to normal. Recovered.
		—†	—	—		
	Serum	—	—	—		
	Influenza	4/2	.017	4.69	500 "	
	complica-	4/4	.063	44.97	500 "	
	tions	—	—	—		
		4/30	.045	22.88		

*Single urine specimen.
†Time elapsed.

0.02 to 0.03 mg. per cc. of this substance or a total of 15 to 30 mg. per 24 hours.

According to Harris and Ray, a peak of saturation may be reached normally within 3 to 5 hours following a very large dose of cevitamic acid. We found that this did not occur in most patients suffering from pneumonia. Very large doses for 3 to 4 days were occasionally required before a peak was reached. In some cases (Table I, Case 2) saturation was not observed even after continued dosing for 8 to 10 days. In all cases when administration of the vitamin was stopped an immediate drop was noted. A second peak could be obtained by an additional relatively small dose (Chart 1).

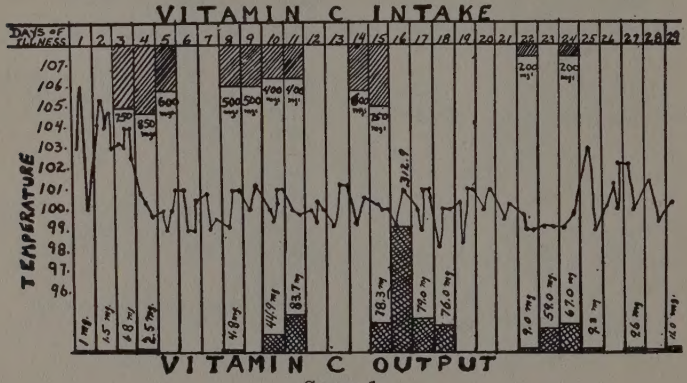

CHART 1.

This fact suggests that at least a portion of the reducing substance in the urine is present as a form of cevitamic acid, and that the overflow results from the saturation of the tissues by the vitamin.

Our experience with 29 pneumonia patients is in accordance with that of Schroeder,[9] who found that vitamin C excretion is decreased in pneumonia. He also found it diminished in cystitis, typhus, and tuberculosis. In our patient with tuberculosis the vitamin C excretion was reduced but on prolonged dosage a high peak was reached which rapidly fell when dosing was discontinued. (Table II, Case 4.) According to Harris,[10] the decrease in vitamin C excretion in pneumonia patients may be due to the increased metabolic rate. Most of our patients were undernourished by reason of poverty.

A preliminary report[11] was given by us on the study of urinary excretion of vitamin C in 10 cases of pneumonia. For our present

[9] Schroeder, *Klin. Woch.*, 1935, 484.

[10] By private communication from L. J. Harris.

[11] Harde, E., Rothstein, I. A., and Ratish, H. D., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1088.

TABLE II.
Cases Other Than Pneumonia.

Case No.	Diagnosis	Date	Mg. cevitamic acid excretion per cc. per diem	Mg. cevitamic acid excretion per diem	Dosage Cevitamic acid*	Remarks
1	Influenza	3/29	.050	23.75		Normal output. Recovered.
		3/30	.050	11.25		
		4/1	.065	31.20		
		4/2	.050	17.16	200 mg.	
		4/3	.075	30.00	100 "	
2	Serum Sickness	5/8	.008	6.50	600 mg. 3 doses	Recovered.
		5/9	.020	13.80	"	
		5/10	.017	12.25	"	
		5/11	.018	9.25	"	
		5/14	.000	0.00	"	
3	Amputation of leg caused by gangrene	5/10	.035	15.75	8 oz. orange juice daily	5/11 before amputation. 5/16 after amputation. Died.
		5/11	.07	15.75		
		—	—	—		
		—	—	—		
		5/16	.000	0.00		
		5/18	.026	3.90		
		5/20	.000	0.00		
		5/21	.025	3.75		
4	Tuberculosis. Pneumonia Type VI	2/27	.024	21.45	6 oranges	Died.
		2/28	.024	40.35	"	
		†	†	†	†	
		3/19	.068	49.80	3/18-600 mg.	
		3/20	.40	200.00	3/19-600 mg.	
		†	†	†	†	
		4/9	.022	14.61‡	Dosing discontinued	
		4/10	1 P.M.—.03	3.00		
			4 P.M.—.00	0.00		
		†	†	†	†	
		4/12	.35	167.50	4/11 to 4/12 750 mg. in 3 doses	
		4/16	1 P.M.—.011	1.10		
			8 P.M.—.00	0.00		
5	Arthritis	2/22	.033	10.14		Recovered.
		2/23	.009	2.65		
		—	—	—		
		—	—	—		
		3/4	.15	22.50		
		3/5	.11	86.10	600 mg. 3 doses	
6	Rheumatic Fever	3/14	.060	24.52	No dosing	Patient reports 1 pint orange juice daily for past 2 years.
		—	—	91.75		
		3/19	.10			
7	Coryza	4/3	.075	34.38	600 mg. 4 doses	Recovered.
		4/4	.10	133.65		
		4/5	.10	55.60		

*Both Merck & Co. and the Hoffman-LaRoche Co. generously supplied the pure cevitamic acid used in these studies.

†Time elapsed.

‡Note reduction in excretion.

work the vitamin C excretion of 38 instances of various pathological conditions were studied. The technique of Harris and Ray[12] was followed. This method is based on the titration of the vitamin C against the dye, 2-6 dichlorophenolindophenol. (Modification of Tillman's Method.) The urine was titrated against .02-0.1 cc. of the dye.

Fresh solutions of the dye were prepared every 24 hours. Occasionally the dye was kept for 48 hours. In this case, it was standardized every 24 hours. The dye solution was kept in an amber bottle, which was placed in the refrigerator (50°F.) whenever it was not in use. The dye was standardized against a solution of 10 mg. of pure cevitamic acid in 50 cc. of 10% acetic acid. The urines, as voided, were collected in bottles containing 15 cc. of glacial acetic acid and placed, as soon as possible, in the refrigerator. Only titrations made within 12 hours are considered in this report, because Harris and Ray reported that urines preserved by 10% acetic acid will retain vitamin C for 10-12 hours.‡

We have occasionally had difficulty in determining the end point in highly colored urine, even when such urines were diluted as recommended by Harris and Ray.[12]

Doses of cevitamic acid were given in quantities varying from 100 mg. to 1 gm. depending upon age and vitamin excretion of the patient.

The urinary titration of cevitamic acid has confirmed previous work with tissues of experimental animals, namely, the diminution of vitamin C in the course of many intoxications and pathological conditions. These states do not suggest scurvy and may be designated hypovitaminosis accompanying, or etiologically related to the pathological conditions with which they occur.

In the pneumonias studied we have been unable to determine any striking correlation between the clinical condition of the patients and their vitamin C excretion. In the greater number of our cases it was noted that when the temperature was high the excretion was low. When saturation occurred it was very often during a drop in the temperature. This seems to support the contention of Harris that increased metabolism is associated with increased destruction of the vitamin in the tissues. The pulse rate showed no significant change. In many of the convalescent cases excretion of vitamin C

[12] Harris, L. J., and Ray, S. N., *Lancet*, 1935, 228.

‡ We have found it impossible to have an exact 10% solution of acetic acid as recommended by Harris and Ray. Experiments with various quantities of urine have shown that the addition of 15 cc. of the glacial acetic acid to the specimen yield approximately a 10% solution.

was low. In one case (Table II, Case 1) with an ordinary balanced hospital diet there was a normal excretion in spite of administering large amounts of vitamin C. This may have been due to the restoration of vitamin C to the tissues depleted during illness. At no time did we note an 80% excretion of the vitamin C intake as described by Harris and Ray for normal individuals.

We have found that administration of crystalline cevitamic acid is preferable to the large amounts of orange juice required to furnish an equivalent quantity of vitamin C, as orange juice in very large quantities may cause diuresis and diarrhea.

Summary. Hypovitaminosis, as determined by delayed saturation, occurs while the temperature is elevated and especially during high fever in pneumonia. Urinary examination after oral administration of large quantities of cevitamic acid in divided doses permits rapid determination of the degree of saturation of the tissues.

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Color Reactions of Keturonic Acids and a Color Test Differentiating α- and β-Glucosides.*

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Color reactions of the keturonic acids have recently become of importance to biology and medicine. In previous papers we have shown that a series of these acids can be prepared from carbohydrates by oxidation of their aqueous solutions with bromine.[1, 2] The solutions of the keturonic acids used in the experiments reported here were prepared as follows:

1% solutions of pure carbohydrates were placed in glass-stoppered flasks together with enough bromine to provide a small excess of liquid bromine throughout the experiments. These mixtures were kept in the dark for 42 days at 25°C. They were then aerated with washed air (and at times with carbon dioxide) to remove the excess bromine and then neutralized with potassium hydroxide to pH 7,

* Aided by a grant from the Research Appropriation of the University of Oklahoma Medical School.

1 Everett, M. R., Edwards, B. G., and Sheppard, F., *J. Biol. Chem.*, 1934, **104**, 11.

2 Sheppard, F., and Everett, M. R., *J. Biol. Chem.*, 1934, **105**, lxxx.

using a spot plate. The color tests were applied to aliquots, with the results given in Table I.

TABLE I.

Color Tests After Oxidation of 1% Solutions with Bromine for 6 Weeks at 25° C.

Carbohydrate	Molisch	Naphtho-resorcinol	Bial	Tashiro-Tietz	Selivan-off	Ferric Chloride
Glycerol	G	O[1]	LB	N	N	P
i-Erythritol	G	LP	GBr	O	N	LP
Adonitol	P	LR	G	P	LGBr	LBr
l-Arabitol	R	R	DG	OR	LGY	RBr
d-Sorbitol	P	Y[1]	O:Gp	P	R	Br
d-Mannitol	P	Y[1]	O:Gp	P	R	Br
Dulcitol	P	LY[1]	O:Gp	P	R	LBr
Perseitol	P	LBr	DG	P	R	LBr
Inositol	GBr	OR	N	N	N	DP:Br
d-Xylose	GBr	LP	N	N	N	B:P
d-Lyxose	G	LP	GBr	N	N	GaBr
l-Arabinose	GBr	LR	N	N	N	LG
l-Fucose	P	DB	G[4]	N	N	Br
l-Rhamnose	R:P	DB	G[4]	N	N	Br
d-Sorbose	P	G	O:Gp[4]	R	R	Br
d-Fructose	P	Y[1]	O:Gp[4]	P	R	Br
d-Glucose	R:P	DB	G	N	N	Br
d-Mannose	P	B	LG	N	N	LPBr
d-Galactose	R:P	B	LG	N	N	Y
d-Glucosamine	G:Ga[2]	LP[6]	N	N	N	LBr
d-Mannoketoheptose	P	Y	P:Gp[7]	P	LBr:R	Y
α-d-Glucoheptose	P	LP	N	R	LR	Y
α-d-Mannoheptose	P	G	LG	R	R	Y
β-Methylxyloside	GBr[3]	R	N	N[8]	N	B:P
α-Methylglucoside	BP[3]	LR	N:LG	N	N	Br
β-Methylglucoside	R:P[3]	DB	G	N	N	LBr
α-Methylmannoside	BP[3]	LR	O:G	N	N	LBr
Cellobiose	R:P	DB	G	N	N	Ga
Lactose	R	DB	G	N	N	Y
Maltose	P	Ga	N:LG	N	N	LBr
Sucrose	P	G	O:Gp[4]	P	R	LBr
Trehalose	P	LP	N:LG	N	N	Br
Melezitose	P	GaB	O:Gp[4]	P	R	Br
Raffinose	P	Br	O:Gp[4]	P	R	Y
Xylan	P	LP	G	N	N	Y
Inulin[5]	R:P	Y[1]	O:Gp[4]	P	R	Br
Dextrin[5]	P	LP	LG	N	N	Y
Soluble Starch[5]	P	LP	G	N	N	Br
Starch[5]	P	LR	G	N	N	Br
Glycogen[5]	P	LP	N	N	N	Br
α-Glucosan	P[7]	B[6]	G[6]	N[7]	N[8]	Br
Levoglucosan	P[7]	LR[6]	DG[6]	R[6]	N[7]	LBr
l-Ascorbic Acid	G:Br[3]	LR[7]	N:LG[7]	N[7]	N[7]	Y
Saccharic Acid	N	LP	N	N	N	Y
Mucic Acid	N	LR	N	N	N	Y

Symbols: B, blue; Br, brown; D, deep; G, green; Ga, gray; L, light; N, negative; O, orange; P, purple; p, precipitate; R, red; Y, yellow; :, upon standing changes to; 1, green in aqueous layer; 2, at 4 days P, original sugar N; 3, original sugar RP; 4, original sugar O:Gp; 5, oxidized sugar gives no color with iodine; 6, original sugar N; 7, original sugar same; 8, original sugar R.

Results obtained too late for tabulation: Oxidized d-ribose and d-arabinose give the same colors as l-arabinose; d-gulose as d-mannose; gentiobiose as lactose; melibiose as trehalose; and tartaric acid as saccharic acid, except a GBr Molisch and Br naphthoresorcinol test with tartaric acid.

In the ferric chloride test of Fenton and Jones[3] we added one drop of 10% ferric chloride solution and 3 drops of 10% potassium hydroxide solution to 5 cc. of neutral sugar solution. The purple color obtained from oxidized glycerol solution indicates that hydroxypyruvic acid is formed[3] and the similar colors from oxidized erythritol, inositol and the pentoses suggest similar products in these cases. These keturonic acids, and also those from glucosamine and ascorbic acid, give green Molisch[4] tests. The Molisch colors from oxidized α- and β-methylhexosides are distinctly different.

Oxidation products from triite and tetrite alcohols give no ketose reactions; those from pentites, the Tashiro-Tietz reaction[4]; those from hexites and heptites give both the Selivanoff[4] and Tashiro-Tietz reactions. Only keturonic acids from aldoheptoses give positive ketose reactions. Oxidized ketoses, together with their oligo- and polysaccharides, still give positive ketose reactions. The behavior of glucosans and ascorbic acid with these reagents is noteworthy.

Many keturonic acids give Bial's test,[4] but not those from pentoses, glucosamine, glucoheptose or α-glucosides. Oxidized ketoses behave like original ones, giving late green precipitates, and oxidized hexite alcohols act similarly. Intense colors are given by oxidized levoglucosan, perseitol and mannoketoheptose.

Classical red or purple colors are given by most keturonic acids in the naphthoresorcinol test of Neuberg and Kobel,[5] while oxidized ketoses and hexites give yellow to green colors. Especially interesting are the deep blue colors given by oxidized methylpentoses, aldohexoses and β-hexosides. Since α-hexosides do not give a similar blue color, we have devised the following rapid differentiating test: Heat 1% methylhexoside solution with excess bromine 4 hours at 65°C., using a flask with ground-in 6 ft. reflux condenser surrounded by an ice-salt cooling mixture. Remove the excess bromine by aeration and neutralize the solution to pH 7 with potassium hydroxide. Only β-hexosides so treated give a deep blue naphthoresorcinol test. α- and β-disaccharide hexosides show much better differentiation by oxidizing them with bromine at 25°C. for 6 weeks as described in this paper. This reaction is limited to hexosides.

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[3] Fenton, H. J. H., and Jones, H., *J. Chem. Soc.*, 1900, **77**, 72.

[4] Hawk, P. B., and Bergeim, O., Practical Physiological Chemistry, P. Blakiston's Son and Co., Philadelphia, 10th Edition, 1931.

[5] Neuberg, C., and Kobel, M., *Biochem. Z.*, 1931, **243**, 435. (We used 2 N hydrochloric acid and ether.)

8479 C

Study of Cholesterol Fractions in Acute Infections of Infants With and Without Eczema.

A. V. Stoesser. (Introduced by Irvine McQuarrie.)

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A study of the plasma cholesterol fractions in acute infections has been reported.[1] This previous investigation included 12 children ranging in age from 18 months to 13 years. It was observed that the total cholesterol values were much less at the height of the infection than during the period of convalescence and that this reduction was due to a marked fall in the ester cholesterol. The free cholesterol changed very little.

Repeatedly, however, reference has been made to the statement that the response of the blood lipids to various factors might not be the same in the infant as in the older child. Plasma cholesterol is considered to be quite low in many infants.[2] Furthermore, a recent study has shown that infants with eczema tend to have a low serum cholesterol.[3] The influence of acute infection upon the already low values of cholesterol in normal and eczematous babies has not been recorded.

Fourteen infants ranging in age from 3 months to 13 months were chosen for this study. Six of the babies had infections of the upper respiratory tract but no eczema. Three had eczema and repeated acute respiratory infections. The remainder did not have eczema but were ill with pneumonia which represents a rather severe form of infection in the infant. The first blood sample was collected during the height of the disease. The second sample of blood was obtained during the period of recovery after the seventh day of normal temperature. All blood samples were drawn between 12 and 16 hours after a meal. Bloor's methods were followed to determine the total, ester, and free cholesterol values.[4, 5, 6]

The results are summarized in Tables I and II.

The total cholesterol values of the infants studied are lower in the acute infection than during the period of convalescence. This

1 Stoesser, A. V., Proc. Soc. Exp. Biol. and Med., 1935, **32**, 1324.

2 Peters, John P., and Van Slyke, Donald, Quantitative Clinical Chemistry, Baltimore, Williams & Wilkins Company, 1931, Vol. 1.

3 Hansen, A. E., Proc. Soc. Exp. Biol. and Med., 1933, **30**, 1198.

4 Bloor, W. R., *J. Biol. Chem.*, 1916, **24**, 227.

5 Bloor, W. R., and Knudson, Arthur, *J. Biol. Chem.*, 1916, **27**, 107.

6 Bloor, W. R., personal communication to the author.

TABLE I.

Plasma Cholesterol Fractions in Infections of Upper Respiratory Tract and in Pneumonia of Infants without Eczema.

Case No.		Total cholesterol		Cholesterol esters		Free cholesterol	
		Mg. per 100 cc. serum.					
A	B	A	B	A	B	A	B
			Height of disease.				
1	8	123	95	56	47	57	48
2	9	118	133	71	91	47	42
3	10	143	92	100	43	43	49
5	11	111	87	59	40	52	47
6	12	104	90	47	37	57	53
7		118		71		47	
Aver.		119	99	69	51	50	48
			Period of convalescence.				
1	8	175	196	115	133	60	63
2	9	138	185	83	125	55	60
3	10	175	185	123	132	52	53
5	11	133	158	74	105	59	53
6	12	138	170	77	125	61	45
7		175		133		42	
Aver.		155	178	100	122	55	55

A—Infections of Upper Respiratory Tract.
B—Pneumonia.

TABLE II.

Plasma Cholesterol Fractions in Acute Infections of Respiratory Tract of Infants with Eczema.

Case No.	Total cholesterol	Cholesterol esters	Free cholesterol
	Mg. per 100 cc. serum		
	Height of infection		
1	143	90	53
2	137	90	47
2	129	80	49
3	114	59	55
3	137	76	61
Aver.	132	78	54
	Afebrile periods		
1	181	105	76
1	177	111	66
2	173	106	67
3	133	64	69
3	153	90	63
3	133	77	56
Aver.	158	92	66

reduction is almost entirely due to a decrease in the ester cholesterol. The changes which occur do not appear to be as marked in the infant as in the older child. This is especially true of the eczema cases. However, in the babies with pneumonia the cholesterol fell during the height of the illness below 100 mg. per 100 cc. in the majority of the cases, and returned to much higher levels during recovery.

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Iodine Number of Serum Fatty Acids in Acute Infections of Infants With and Without Eczema.

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The changes which take place in the cholesterol content of the serum during the acute infections occurring in the infants observed in the preceding paper[1] are accompanied by a fall in the plasma total fatty acids. The serum unsaturated fatty acids have already been found to be replaced in part by more and more saturated acids during acute respiratory infections in older children.[2] In infants the observation has been made that eczema is associated with an abnormally low serum content of unsaturated fatty acids[3] but no study has been recorded of the influence of acute infection on the unsaturation of the serum fatty acids.

This paper presents the observation made in 14 infants with acute illnesses. They are the same babies which were chosen for the cholesterol study. All blood samples obtained during and after the height of each infection were drawn between 12 and 16 hours after a meal. The first blood sample was collected as soon as possible after the infant was admitted to the hospital at the height of the disease and the second blood sample after the seventh day of convalescence. Bloor's methods[4] were followed to determine the blood lipids. The Rosenmund-Kuhnhenn method[5] as modified by Page, Pasternak and Burt[6] was used to determine the iodine absorption of the serum fatty acids.

The results are summarized in Tables I and II.

The fall in plasma total fatty acids which takes place during the height of acute infections in infants is paralleled by a drop in the iodine absorption values. This signifies that in most instances the iodine number of serum fatty acids is definitely lower in the febrile period of the disease than during the period of recovery. Acute infection produces in the infant the same changes previously found to occur in the older child. The serum fatty acids are less

[1] Stoesser, A. V., Proc. Soc. Exp. Biol. and Med., 1936, **34**, 10.

[2] Stoesser, A. V., Proc. Soc. Exp. Biol. and Med., 1935, **32**, 1326.

[3] Hansen, A. E., Proc. Soc. Exp. Biol. and Med., 1933, **30**, 1198.

[4] Bloor, W. R., *J. Biol. Chem.*, 1928, **77**, 53.

[5] Rosenmund, K. W., and Kuhnhenn, W., *Z. f. unter. d. Nahr. d. Nahr. u. Genuss.*, 1923, **46**, 154.

[6] Page, H. H., Pasternak, L., and Burt, M. L., *Biochem. Z.*, 1930, **223**, 445.

TABLE I.

Iodine Numbers of Serum Fatty Acids in Infections of Upper Respiratory Tract and in Pneumonia of Infants without Eczema.

Case No.		Total fatty acids Mg. per 100 cc. serum		Iodine absorbed Mg. per 100 cc. serum		Iodine number	
A	B	A	B	A	B	A	B
				Height of disease			
1	8	426	304	416	252	97	82
2	9	278	323	303	256	108	79
3	10	390	311	350	289	89	92
5	11	312	274	229	248	73	90
6	12	257	304	234	246	91	80
7		414		356		85	
Aver.		346	303	314	258	90	84
				Period of convalescence			
1	8	471	342	545	332	113	97
2	9	350	373	413	358	118	96
3	10	448	335	463	433	103	129
5	11	306	406	255	475	83	117
6	12	257	361	263	435	102	120
7		457		435		95	
Aver.		381	363	395	406	102	111

A—Infections of Upper Respiratory Tract.
B—Pneumonia.

TABLE II.

Iodine Numbers of Serum Fatty Acids in Acute Infections of Respiratory Tract of Infants with Eczema.

Case No.	Total fatty acids Mg. per 100 cc. serum	Iodine absorbed Mg. per 100 cc. serum	Iodine number
	Height of infection		
1	316	258	81
2	375	403	107
2	395	341	86
3	501	418	83
3	397	373	93
Aver.	396	358	90
	Afebrile periods		
1	402	364	90
1	342	331	97
2	432	389	90
3	513	488	95
3	444	467	105
3	540	600	111
Aver.	445	439	98

unsaturated during an acute illness. The already moderately low iodine number of serum fatty acids in the eczematous babies is still more reduced by acute respiratory disease, and there is a tendency for it to remain at low levels for some time. This slow return to higher levels was also observed in the infants without eczema. However, the pneumonia cases had a more rapid fall in the iodine

number with a rather quick return to normal levels after the pneumonia had completely resolved, leaving no complications.

Incidentally a very interesting observation was made in connection with eczematous babies. During the first half of the acute illness the eczema improved remarkably although no special treatment of the skin was instituted. Then at the end of the febrile period, the skin became worse, remaining in this condition for weeks. This period of improvement may coincide with the sudden flood of unsaturated fatty acids into the blood stream which Boyd[7] claims occurs at onset of fever. In one instance an eczematous infant fairly well under control developed an acute illness with fever, and the eczema became worse without the initial phase of improvement. The effect of acute infections on the course of infantile eczema is the subject of further investigations which are in progress.

8481 C

Water Metabolism of the Rat Following Removal of the Anterior Lobe of the Hypophysis.*

RICHARD I. PENCHARZ, JAMES HOPPER, JR., AND EDWARD H. RYNEARSON. (Introduced by H. M. Evans.)†

From the Institute of Experimental Biology, University of California, and the Division of Medicine, The Mayo Clinic, Rochester, Minn.

There is considerable evidence that experimental diabetes insipidus is neither strictly an endocrine disturbance nor entirely an involvement of the brain but probably the result of both. Rats can be rendered diabetic by removal of the posterior lobe or by a stab wound in the base of the brain, providing the latter injury is such that it completely severs the stalk from its attachment to the brain.[1,2]

Fisher, Ingram and Ranson,[3] availing themselves of the Horsley-

[7] Boyd, E. M., *Canadian Med. Assn. J.*, 1935, **32**, 500.

* Aided by a grant for the study of the metabolic relations of the anterior hypophysis contributed by Robert R. Presnell, Frank Tuttle, Arthur Stebbins, and Mrs. Gordon Kahn, of Los Angeles.

† The authors wish to thank Miss Frances Dobell for her valuable assistance in this work.

[1] Richter, C. P., *Am. J. Physiol.*, 1933, **106**, 80.

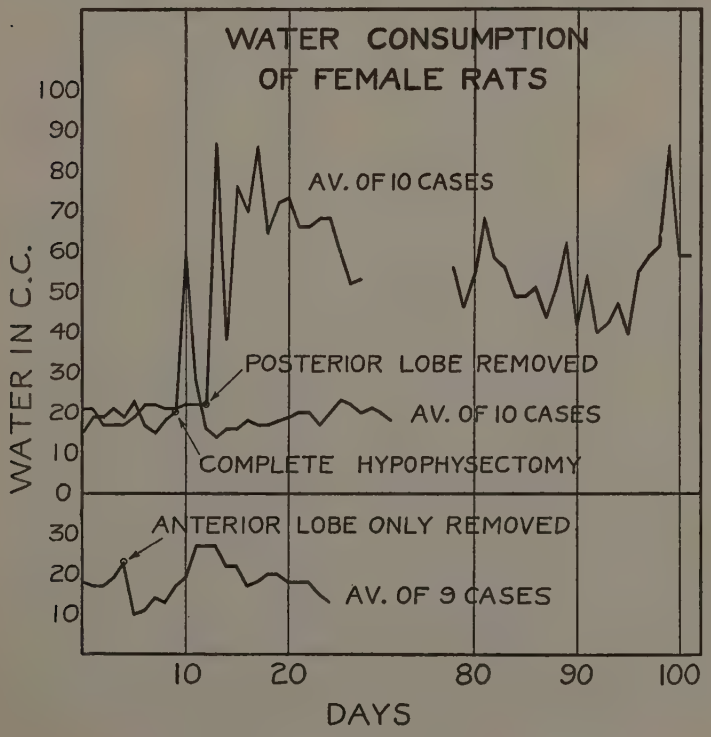
[2] Richter, C. P., *Am. J. Physiol.*, 1934, **110**, 439.

[3] Fisher, C., Ingram, W. R., and Ranson, S. W., *Arch. Neurol. and Psychiat.*, 1935, **34**, 124.

Clark stereotaxic instrument, investigated the effect of restricted hypothalamic lesions in the cat. Their observations were combined with careful histological studies of the resulting degeneration and they were able to demonstrate that a permanent diabetes insipidus ensued only when there was complete interruption of the hypothalamico-hypophyseal tract.

The literature contains numerous reports on the effect of complete hypophysectomy, hypophyseal injuries, and lesions of the tuber cinereum and the probable relation of these to experimental diabetes insipidus, but the writers are not aware of any investigations in which an effort was made to determine the effect of removal of the anterior lobe without concomitant injury to the posterior lobe or stalk.

The experimental material included 47 female rats, varying in age from 90 to 120 days at the time of operation. The results secured with completely hypophysectomized rats, rats with the posterior lobe alone removed, and the rats with the anterior lobe re-



moved, are summarized in the accompanying chart. It will be noted that following complete hypophysectomy there is an immediate and transitory polydipsia, a condition now known for some time.[4] Removal of the posterior lobe, providing sufficient anterior lobe tissue has been left behind, invariably produces a pronounced increase in water consumption which appears to be permanent. The results summarized in this chart include only those animals in which there was minimal injury to the anterior lobe. Findings of more extreme injuries to this portion of the gland varied somewhat from those described above and will be related elsewhere.

What we especially wish to direct attention to in this report is the result obtained by the removal of the anterior lobe alone. In contrast to the condition secured following total hypophysectomy or removal of the posterior lobe, water intake of rats following ablation of the anterior lobe falls below the normal or preoperative level. This decreased consumption of water is maintained for about a week; at the end of that time it returns to the normal level.

From our own experiments and from those of Richter, it would appear that either a surviving portion of tissue of the anterior lobe, in the absence of the posterior lobe, or section of the stalk is a necessary condition for the production of a permanent diabetes insipidus. If the polydipsia is provoked by a diuretic principle originating in the anterior lobe, one would expect a similar disturbance in the metabolism of water following implantation of fresh rat anterior lobe tissue in the hypophysectomized animal. With this in mind, 6 hypophysectomized rats were implanted with fresh rat anterior lobe tissue. Each rat received from one to 3 anterior lobes every other day for a period of 20 days. The results were completely negative. The intake and output of water by these animals remained unaffected and never rose above normal levels.

Richter suggests that the loss of cerebrospinal fluid, incidental to the removal of the pituitary, might be a contributing factor in the production of diabetes insipidus. It should be pointed out that in our operations such loss of fluid was not observed; however, to check this possibility the cerebrospinal fluid of 12 rats was drained through a small opening in the base of the skull posterior to the pituitary and the intake of water and output of urine were measured daily for a period of several weeks. No disturbance in the metabolism of water appeared.

Summary. 1. Complete hypophysectomy is followed by transient polydipsia and polyuria which disappear within 24-36 hours after

[4] Aschner, B., *Pflüg. Arch. f. Physiol.*, 1912, **65**, 341.

the operation. 2. Removal of the posterior lobe, with as little injury to the anterior lobe as possible, is invariably followed by a marked increase in the water intake and water output. This condition is apparently permanent, for it can still be observed 6 months or more after the operation. 3. Ablation of the anterior lobe, leaving only the pars nervosa and intermediate portion intact, including the stalk attachment to the brain, produces a slight decrease in the water intake for about a week after the operation. At the end of this time the water consumption returns to the normal level. 4. Hypophysectomized rats implanted with anterior lobes of the rat hypophysis show no disturbance in water metabolism. The water intake and output of animals so treated, even over a period of 20 days, never rise above those observed in the normal animal.

8482 C

A Method for Determination of Total Pigment in Bile Which Is Applicable to "Biliverdin Biles."

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Methods for quantitation of bile pigments, with the exception of the methods of Hooper and Whipple,[1] Beccari,[2] Kerppola and Leikola,[3] and Peterman and Cooley[8] have been confined to modifications of the van den Bergh method[4] for the determination of bilirubin. That the pigment in the bile of carnivora is present chiefly as bilirubin and that of herbivora as biliverdin[5] is a conception based partly upon physical appearances of the respective bile and partly upon specific tests for these particular constituents. Modifications of the van den Bergh method are satisfactory for clinical identification or quantitation of bilirubin; however, such methods are without value when used for the quantitation of pigment in bile in which the pigment is present in the form of oxidized bilirubin

[1] Hooper and Whipple, *Am. J. Physiol.*, 1916, **40**, 332.

[2] Beccari, *Boll. Soc. Ital. Biol. Sper.*, 1928, **3**, 332.

[3] Kerppola and Leikola, *Skan. Arch. Physiol.*, 1929, **55**, 70.

[4] McNee, *Quart. J. Med.*, 1923, **16**, 390.

[5] Hawk and Bergeim, Practical Physiological Chemistry, Philadelphia, 1927, 10th edition, p. 329.

derivatives. Furthermore, Müller and Engel[6] have shown spectrophotometrically that the diazo reaction of the van den Bergh test detects only 30 to 60% of the bilirubin present in a solution, while Beccari[7] has reported that a number of compounds other than bilirubin, and present both in bile and serum, gave color reactions which were indistinguishable from that given by bilirubin.

In attempting to use the method of Hooper and Whipple[1] for the determination of total pigment, it was found that the method was uncertain and has objectionable features which have been pointed out by Peterman and Cooley.[8] These investigators have shown that the blue-green color taken as an end-point in the Hooper-Whipple and their own methods is a mixture of unoxidized bilirubin and bilicyanin, the combination of which constitutes the green pigment of bile heretofore considered as an individual pigment, "biliverdin." Thus, in colorimetric determinations, using a blue filter disc, only that portion of bilirubin which has been oxidized to bilicyanin is estimated. In order that the blue-green color be obtained always it is necessary that the amount of oxidizing reagent used and the time required to develop maximum color intensity be varied according to the amount and character of the pigment present. In our hands, the amount of nitric acid suggested by Hooper and Whipple proved sufficient to oxidize the pigment in a number of samples of dogs' bile to a greenish-yellow color within 12 hours instead of 24 hours. Thus, it was necessary to adapt the method to each sample of bile before it could be applied with any degree of reliability. Once adapted to a sample of bile, our experimental error with this method of analysis was from 5 to 10%.[9] Further, we desired to assay the total pigment in bird and rabbit bile, to which the Hooper-Whipple and Peterman-Cooley methods are not applicable.

Attempts to obtain a method applicable to both bilirubin and "biliverdin" bile resulted in the following method. The procedure is based upon the oxidation of the major pigments to yellow pigments which are soluble and relatively stable. Maximum intensity of the orange-yellow coloration is obtained in 16 hours, fading gradually to pale-yellow within 36 hours. The procedure is as follows: One-half or one cc. of bile is pipetted (attention being given to uniform drainage) into a 15 cc. volumetric tube and mixed with 10 cc. of a 1:1 glacial acetic acid-95% alcohol mixture. One cc. of freshly

[6] Müller and Engel, *Klin. Wochnschr.*, 1930, **9**, 2305.

[7] Beccari, *Klin. Wochnschr.*, **5**, 671.

[8] Peterman and Cooley, *J. Lab. and Clin. Med.*, 1933, **19**, 723, 743.

[9] Scribhishaj, Hawkins and Whipple, *Am. J. Physiol.*, 1931, **96**, 449.

prepared 2% *ammonium persulphate* solution is added and the volume made up to 15 cc. with acid-alcohol reagent. After shaking to insure thorough mixing of the contents, the tubes are stoppered and allowed to stand in a constant temperature room (25 to 30°C.) for from 16 to 24 hours when they are compared directly in a colorimeter against a bilirubin standard which has been treated in exactly the same manner. Determinations are made in duplicate. Temperatures higher than 30° are inadvisable since the products of oxidation are unstable with heat, and although the reaction can be greatly hastened by heating such practice is conducive to inaccurate results. Since sun light is known to influence the oxidation of bilirubin in acid solution, the oxidation should be carried out in a dark room. A chloroform solution of Eastman's bilirubin is used as standard, assuming the preparation to be 100% pure. Five cc. of this solution, containing from 1 to 2 mg. bilirubin, are pipetted into a volumetric tube and mixed to volume with the acid-alcohol reagent and one cc. 2% ammonium persulphate. A permanent standard has advantages over the bilirubin standard and may be obtained by matching the oxidized bilirubin standard against the following solution: 36 gm. $FeCl_3$, 40 gm. $CoCl_2$, 5 cc. conc. HCl, and 1 cc. 0.05 M $KMnO_4$. This is made up to 2 liters, allowed to stand one day, and filtered.

The selection of the glacial acetic acid-95% alcohol solvent and ammonium persulphate as oxidizing reagent was the result of an extensive study of the oxidative reactions of pigments in bile with a number of the commonly used oxidizing reagents. From our studies we concluded that, under the chosen conditions, the oxidation of bilirubin occurred uniformly through graded steps of color change. The oxidation products appear more and more stable as each successive stage is reached; thus, in ammonium persulphate we have an oxidizing reagent that may be present in excess, within limits, without the marked acceleration of the oxidative reactions obtained by excess amounts of more labile oxidizing reagents.

We found it convenient to read the permanent standard against the standard bilirubin solution at 16, 18, 20, and 22 hours and in this way established a bilirubin equivalent for each corresponding time interval. Within 8 hours after the addition of the oxidizing reagent to the acid-alcohol solution of bile an orange-red color develops, and at 16 hours the solution is deep orange-yellow. Between 18 and 22 hours the color change is scarcely perceptible but at 26 hours there is an accelerated transition of color from orange-yellow to pale-yellow. Eighteen or 20 hours have been selected as standard

for the time of color comparison with the permanent standard solution. At this time the coloration is in the middle of the plateau for this stage of color change and the values obtained on a sample of bile when read against a bilirubin standard are the same at 18 and 20 hours, indicating that the standard and the sample are being oxidized at the same rate.

An excess of oxidizing reagent, that is 1 cc. of a 2% solution, is specified because it was found to give more uniform comparative results when applied to a wide variety of bile samples. Chicken and pigeon bile, and some samples of dog bile, when treated by this procedure using a 1% solution of oxidizing reagent show the presence of a bluish pigment when the reading in a colorimeter is taken. This pigment, probably bilicyanin, is readily oxidized to orange-yellow when 2% persulphate is employed, yet the oxidizing reaction is not increased to the extent that the oxidation is carried out of the orange-yellow color phase within 22 hours.

Although a white precipitate forms, there is no apparent adsorption of pigment and the precipitate is filtered off preparatory to colorimeter readings.

In order to test the accuracy of the method, the recovery of added bilirubin to dog bile was investigated with the typical results shown in Table I. The bilirubin was added in chloroform solution. Control determinations on samples of bile to which pigment-free chloroform was added showed that this solvent has no effect on the oxidation or the colorimetric readings.

The method proved accurate to (±)0.02 mg. pigment, thus reducing the experimental error to less than 2%.

Total pigment in bile, as determined by the above procedure, was compared with the bilirubin content as shown by the van den Bergh

TABLE I.

Bile Sample	cc. Bile	Mg. Added Bilirubin	Mg. Pigment Determined	Mg. Pigment Calculated	Mg. Error	% Error
A	0.5	—	1.240	—	—	—
	0.5	0.104	1.363	1.344	0.019	+1.33
	0.5	0.208	1.430	1.448	0.018	—1.25
	0.5	0.416	1.640	1.656	0.016	—0.91
B	0.5	—	1.000	—	—	—
	0.5	0.104	1.110	1.104	0.006	+0.54
	0.5	0.208	1.190	1.208	0.018	—1.50
	0.5	0.416	1.430	1.416	0.014	+0.98
C	1.0	—	2.000	—	—	—
	1.0	0.080	2.060	2.080	0.020	—0.97
	1.0	0.160	2.144	2.160	0.016	—0.79
	1.0	0.240	2.272	2.240	0.032	+1.42

TABLE II.

Day	Mg. Total Pigment	Mg. Bilirubin van den Bergh	% Bilirubin van den Bergh
Dog X			
1	178.4	77.4	43.3
3	169.9	94.3	55.5
4	299.0	162.1	54.3
5	101.0	43.9	43.2
Dog XV			
1	594.0	284.0	47.8
2	362.8	184.0	50.7
3	155.5	85.0	54.6
4	276.5	145.0	52.4

The above values are in terms of milligrams of pigment per 100 cc. of bile.

method with results in Table II. The bile for these comparative tests was obtained daily from 2 total bile fistula dogs. It is interesting to note that the van den Bergh values when compared with total pigment figures agree favorably with percentages reported by Müller and Engel.[6]

The method has been used rather extensively during the past 18 months for assaying pigment in the bile of birds and mammals.

8483 P

Influence of Water Administration upon Oxygen Consumption Rate in Shock.

Harry A. Davis. (Introduced by E. B. McKinley.)

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The production of water intoxication in human beings is difficult. However, instances of such intoxication have been reported,[1-4] and it has been the subject of experimental investigation in lower animals.[5, 6, 7] Recent studies[8, 9] upon the metabolic effects of water

[1] Larson, E. E., Rowntree, L. G., and Weir, J. F., *Arch. Int. Med.*, 1922, **29**, 306.

[2] Miller, J. L., and Williams, J. L., *Am. J. Med. Sc.*, 1921, **161**, 327.

[3] Trousseau, A., Lectures on Clinical Medicine, London, 1867.

[4] Helwig, F. C., Schutz, C. B., and Curry, D. E., *J. Am. Med. Assn.*, 1935, **104**, 1569.

[5] Rowntree, L. G., *J. Pharmacol. and Exp. Therap.*, 1926, **29**, 135.

[6] Misawa, H., *Jap. J. Med. Sc. Tr. VIII*, 1927, **1**, 355.

[7] Kylin, E., *Z. f. d. ges. exp. Med.*, 1928, **63**, 606.

[8] Davis, H. A., Proc. Soc. Exp. Biol. and Med., 1935, **33**, 242.

[9] Davis, H. A., Proc. Soc. Exp. Biol. and Med., 1935, **33**, 245.

have suggested that a definite relationship exists between the initial metabolic rate and the rise in metabolism induced by water. Following hemorrhage, there is a marked diminution in the metabolic response to water and electrolytes.[10] This study has been undertaken to determine whether or not there is an altered susceptibility to water intoxication in animals subjected to experimental traumatic shock.

Healthy dogs weighing between 6-19 kg. were used. Anesthesia was induced by sodium barbital in a dosage of 250 mg. per kilo of body weight. Blood pressure tracings were made in the usual manner before and during the administration of fluid. The metabolic readings were obtained by a Krogh apparatus before, and at 15-minute intervals after, the production of shock. Shock was produced by: 1. massive hemorrhage to the extent of ⅓ of the calculated blood volume. 2. crushing of the muscles of the hind legs or by prolonged handling of the exposed bowel. 3. ligation of the portal vein. 4. injection of histamine dihydrochloride. The fluid utilized was an isotonic (0.9%) sodium chloride solution heated to 38°C., and given by vein at a constant rate of 16 cc. per minute. The standard amount of solution was 3,000 cc. Ten of the animals were used as controls. The urine output was estimated by a catheter placed in the emptied urinary bladder.

Shock is accompanied by a marked fall in the rate of oxygen consumption. A diminution of 30% to 50% of the original rate was common. This finding is in agreement with the work of other observers.[11, 12] Shock produced by hemorrhage is characterized by a tendency to a rapid and spontaneous recovery, so that the metabolic rate commences to rise within 15 minutes and, after a few hours, may have reached almost its initial level. When severe trauma was involved in the experiment, the metabolic rate tended to remain fixed at a low level and a spontaneous rise was considerably delayed. Following portal vein ligation the fall in metabolism was delayed but was thereafter progressively downward. The fall in the rate of oxygen consumption after the administration of histamine dihydrochloride was long sustained, and a spontaneous rise was slow to occur.

In the normal control animals, administration of water by vein leads to a gradual prolonged rise in the oxygen consumption rate. The maximal increase in metabolism is reached only after 1,400 cc.

[10] Davis, H. A., *Science*, 1935, **81**, 493.

[11] Aub, J. C., and Wu, H., *Am. J. Physiol.*, 1920, **54**, 388.

[12] Henderson, Y., *et al.*, *Am. J. Physiol.*, 1908, **21**, 126.

to 2,400 cc. of fluid have been injected. Thereafter, the rise is sustained. The absolute increase of metabolism is directly proportional to the original rate of metabolism. In shock the maximal metabolic response is reached at an earlier stage of the fluid injection, *i. e.*, after 700 cc. to 1,200 cc. of the solution have been given. Further administration of fluid results in a progressive decrease in the rate of oxygen consumption.

The tolerance of a normal healthy animal for water is great, so that fluid to the extent of 25% of the total body weight of the animal may be given without harmful effects. In shock, the tolerance of the organism is much diminished, inasmuch as the administration of a quantity of water equivalent to only 5% to 12% of the body weight is followed by a fall in the rate of metabolism. Animals suffering from the effects of shock showed a marked reduction in urine secretion. This was most evident where shock was accompanied by a diminution of the total bulk of circulating blood. The administration of water in amounts sufficient to depress the oxygen consumption rate of the shocked animals seemed to delay the establishment of a normal water diuresis.

8484 P

Effect of Total Thyroidectomy on Response to Injection of Adrenalin.

HALL SEELY AND ELLIOTT C. CUTLER.

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Since Elliot's work in 1907, the increased sensitivity to adrenalin in vessels deprived of sympathetic innervation has been well known. Clinical observations on patients following total removal of the thyroid have suggested a close dependency of adrenalin efficiency on the presence of thyroid hormone. The injection of adrenalin in patients deprived of thyroid secretion seemed to produce less vasoconstriction. This diminished action of adrenalin was very striking in limbs rendered especially sensitive to adrenalin by sympathectomy.

To test the accuracy of these observations the following experiments were performed on dogs:

Dogs were trained to stand quietly in a light overhead harness. Skin temperature curves were made at the same time of each fore

leg by means of thermocouples attached to the skin. A number of such skin temperature curves under standard conditions revealed no difference between the fore legs. Being thus assured of a proper control, the sympathetic fibers supplying the left leg were destroyed by the removal of the stellate and each adjoining ganglion above and below. After allowing adequate time for the degeneration of the post-ganglionic fibers, determinations by the thermocouples revealed the customary 4° to 5° difference in temperature between the 2 legs. Skin temperature curves were now taken simultaneously on the 2 forelegs following an intravenous injection of a 1-20,000 solution of adrenalin chloride. The injections caused consistently a much greater and more prolonged fall in temperature on the operated side. When a number of similar curves were accumulated under identical standard conditions, the dogs were subjected to total thyroidectomy. After recovery and during the next 8 months temperature changes in both fore legs were again recorded following adrenalin injection. The post-thyroidectomy curves showed a great loss in the sensitivity of the sympathectomized vessels to adrenalin.

In the dogs deprived of thyroid secretion the former prompt pressor response and resulting abrupt and prolonged fall of tem-

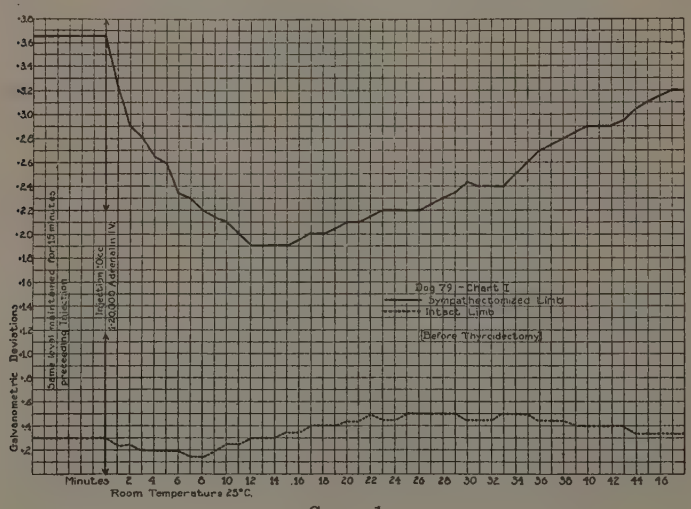

CHART 1.

Curves following injection of 10 cc. of 1-20,000 solution of adrenalin chloride intravenously before thyroidectomy. Note the immediate and prolonged fall of temperature in the sympathectomized limb. The intact limb responds vividly to the injection. The skin temperature changes are plotted in deviation units of the galvanometer used with the thermocouple.

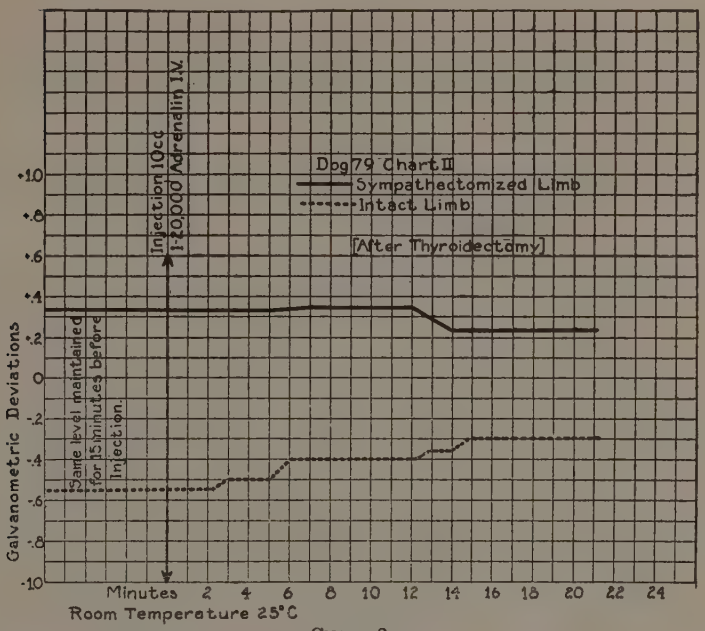

CHART 2.

Curves following total thyroidectomy. Note lack of temperature fall following the injection.

perature on the operated side was almost entirely abolished. The curves for the intact limb were but little changed by total thyroidectomy. These experiments served to confirm our clinical impressions of the direct relationship between the thyroid hormone and the effectiveness of circulating adrenalin.

The accompanying charts on Dog No. 79 are an example of the changes produced.

8485 C

Ovaries of Immature Female Rats Receiving Pregnancy Urine Extract and Combinations of Pregnancy Urine Extract and Oestrin.*

J. M. Wolfe.

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We have previously reported[1] that injections of large amounts of oestrin into mature female rats produced corpora lutea in the ovaries of these animals equal in size to those of pregnant rats. Similar results have been reported by Hohlweg[2] and Selye, Collip and Thomson.[3] Other experiments[3, 4, 5] have demonstrated that injection of oestrin simultaneously with A.P.L. (anterior pituitary-like substance) enhances the luteinizing capacity of A.P.L. and its capacity to increase the ovarian weights of immature rats. Hisaw and associates[6] and Lane[7] have demonstrated that injection of oestrin stimulated the production on the luteinizing hormone in the anterior hypophysis. The data presented below support the view that combined injection of oestrin and A.P.L. exerts a much greater effect on the ovaries of immature female rats than do injections of the A.P.L. factor alone.

In the first series of experiments a group of immature female rats received a single injection of 25 units of A.P.L.† They were killed 10 days later after no further treatment. A second group, littermate sisters to those above, received a single injection of A.P.L. and beginning on the fourth day following the administration of A.P.L. daily injections of 200 units of oestrin,‡ until the tenth day

* These studies were aided by grants from The Grants-in-Aid Committee of the National Research Council and by the Division of Medical Sciences of the Rockefeller Foundation.

1 Wolfe, J. M., Proc. Soc. Exp. Biol. and Med., 1935, **32**, 757.

2 Hohlweg, W., *Klin. Woch.*, 1934, **13**, 93.

3 Selye, H., Collip, J. B., and Thomson, D. L., Proc. Soc. Exp. Biol. and Med., 1934, **32**, 1377.

4 Collip, J. B., *Internat. Clinics*, 1932, **4(41)**, 65.

5 Magath, M. A., and Rosenfeld, R. M., *Arch. ges. Physiol.*, 1933, **233**, 311.

6 Hisaw, F. L., Fevold, H. L., Foster, M. A., and Hellbaum, A. A., *Anat. Rec.*, 1934, **60**, 52 (supplement).

7 Lane, C. E., *Am. J. Physiol.*, 1934, **110**, 681.

† Extracts of pregnancy urine, Follutein, were furnished by E. R. Squibb and Sons through the courtesy of Dr. J. J. Durrett.

‡ Progynon-B, the benzoic acid ester of dihyroestrin, was used. A portion of this material was furnished gratuitously by Dr. Erwin Schwenk of the Schering Corporation.

following the injection of A.P.L., at which time the rats were sacrificed. As controls for these experimental groups a group of untreated littermate sisters were used. In a second series of experiments a group of 31 immature female rats received daily injection of 25 units of A.P.L. for 10 days while a second group received the same amount of A.P.L. for the same length of time and in addition 200 units of oestrin daily for the last 6 days of the total experimental period of 10 days. At autopsy the body, ovary, and pituitary weights of all animals were obtained. After fixation and embedding serial sections of the ovaries and representative sections of the uteri and vaginae were cut and stained. The ovaries of all 4 experimental groups contained corpora lutea; none were found in the ovaries of the control rats. The relative size of the corpora lutea was determined by measuring the largest diameters of the corpora lutea in millimeters with a micrometer eye piece. The product of the 2 great diameters was calculated and the average of these products for the corpora lutea in the ovary was considered the size for the corpora in that ovary. The ovarian weights of the rats of the various groups and the products of the 2 greatest diameters of the corpora lutea are given in the frequency-distribution tables I and II. The mean weights of the ovaries and the mean

TABLE I.

Frequency Distribution Table Giving Range and Mean of Ovarian Weights in Experimental and Control Groups.

Frequency Intervals mg.	Controls	Single inj. 25 u. A.P.L. A.P.L.	Single inj. 25 u. A.P.L. A.P.L.-Oestrin	Daily inj. 25 u. A.P.L. A.P.L.	Daily inj. 25 u. A.P.L. A.P.L.-Oestrin
10- 14.9	1	2	—	—	—
15- 19.9	6	8	—	—	—
20- 24.9	3	5	1	—	—
25- 29.9	—	2	4	—	—
30- 39.9	—	2	4	1	—
40- 49.9	—	—	—	6	—
50- 59.9	—	—	1	6	—
60- 69.9	—	—	—	8	—
70- 79.9	—	—	—	7	1
80- 89.9	—	—	—	2	2
90- 99.9	—	—	—	1	3
100-109.9	—	—	—	—	3
110-119.9	—	—	—	—	6
120-129.9	—	—	—	—	4
130-139.9	—	—	—	—	3
140-149.9	—	—	—	—	3
150-159.9	—	—	—	—	2
160-169.9	—	—	—	—	1
170-179.9	—	—	—	—	—
Mean Wt., Mg.	18.5	21.8	32.7	72.8	119.6
Rats in group	10	19	10	41	28

measurements of the corpora lutea in the various groups are also given.

Table I shows that a single injection of 25 units of A.P.L. only slightly increased the weight of the ovaries. The ovaries of this group usually contained from 1 to 5 corpora which, with 2 exceptions, were very small. In most rats the products of the 2 diameters of the corpora were below 0.4 mm., while the mean for the whole group was 0.5 mm. (Table II.) In the animals in which daily injection of oestrin was given in addition to the single injection of 25 units of A.P.L. the ovaries were considerably increased in weight (Table I). While the corpora lutea were not increased in number they were greatly increased in size. For the whole group the mean product of the 2 diameters of the corpora lutea was 1.2 mm. (Table II), a mean increase in size of 140% when compared with the corpora in the previous group.

TABLE II.

Frequency Distribution Table Giving Range and Mean of the Relative Size (mean product of the greatest diameter) of Corpora Lutea in the Various Groups.

Frequency Intervals mm.	Single inj. 25 u. A.P.L.		Daily inj. 25 u. A.P.L.	
	A.P.L.	A.P.L.-Oestrin	A.P.L.	A.P.L.-Oestrin
.0- .4	10	—	2	—
.5- .9	7	2	29	—
1.0-1.4	2	5	2	28
1.5-1.9	—	3	—	4
2.0-2.4	—	—	—	—
Mean—mm.	.5	1.2	.7	1.3

The ovaries of the rats receiving daily injection of A.P.L. for 10 days were rather markedly increased in weight; the mean weight was 72.8 mg. and the range from 40 to 109 mg. (Table I). Although these ovaries contained many corpora lutea it is a point of considerable interest that their individual size was relatively small; the mean product of the 2 greatest diameters for the whole group was 0.7 mm. and in only 2 rats was this figure above 1 mm. (Table II). The ovaries of the rats which received 10 daily injections of A.P.L. plus daily injection of oestrin for the last 6 days of the experimental period were greatly increased in weight; the mean was 119.6 mg., the range from 70 to 169 mg. (Table I). The corpora lutea in these ovaries were markedly increased in size. The mean product of the 2 diameters for the entire group was 1.3 mm.; the range of variation is given in Table II.

Our studies would indicate that injection of oestrin simultaneously with A.P.L. augments the capacity of A.P.L. to increase the weight of the ovaries of test rats. This weight increase we believe

is due to the increased size of the individual corpora lutea in the ovaries of rats which received oestrin.

8486 C

Effect of a Previous Distention of the Intestine on Reflex Inhibition of Gastric Motility.*

JOE LALICH, R. C. HERRIN AND WALTER J. MEEK.
From the Department of Physiology, University of Wisconsin.

In studying the nerve pathways involved in the reflex inhibition of hunger contractions by distention of a Thiry fistula loop in the dog,[1] it was noticed that a previous long-continued distention of the loop brought about a lower threshold for the reflex.

In the experiments here reported, five animals were prepared with Thiry fistulae in the usual manner, the loops being 15-20 cm. long and taken from the upper ileum. After complete recovery the dogs were trained to swallow a stomach tube and to lie quietly on the table without any restraint. Gastric movements were recorded by the customary balloon-tambour method. The intestinal loops were distended by air inflation of a thin-walled balloon which was over-size relative to the lumen of the gut. The balloon was tied over a glass rod so that the expansion would be entirely lateral and the pressure readings would closely approximate the pressure actually applied to the intestinal wall. All records were made after a fasting period of 18-24 hours. In testing for inhibition of gastric motility the intestinal loops were inflated for a short period, usually 10-30 seconds. In sensitizing or reinforcing the reflex, the loops were subjected to a continuous pressure of 75 mm. Hg. for 24 hours, following which tests were again made.

The essential results may be seen in Table I and Fig. 1. In the control experiments pressures in the loops of 75 to 120 mm. Hg. produced inhibition of gastric movement in 25 out of 55 tests. Each dog responded about half the time, the percentage of responses increasing somewhat with the higher pressures. It was noted that hunger contractions of type 2 were most easily affected. Often there was a marked lowering of tonus with the presence of smaller contractions at this lower level. When pressures below

* Supported in part by the Wisconsin Alumni Research Foundation.

[1] To appear in the *American Journal of Physiology.*

75 mm. Hg. were used there were only 4 gastric inhibitions in 43 tests, one animal now failing to respond at all.

TABLE I.

Occurrence of Gastric Inhibition Produced by a Short Period of Intestinal Distention. Tests Made in 5 Animals.

Controls	After Previous Distention for 24 Hours
Pressures above 75 mm. Hg.	Pressures above 75 mm. Hg.
25 Positive	22 Positive
30 Negative	4 Negative
Pressures below 75 mm. Hg.	Pressures below 75 mm. Hg.
4 Positive	32 Positive
39 Negative	4 Negative

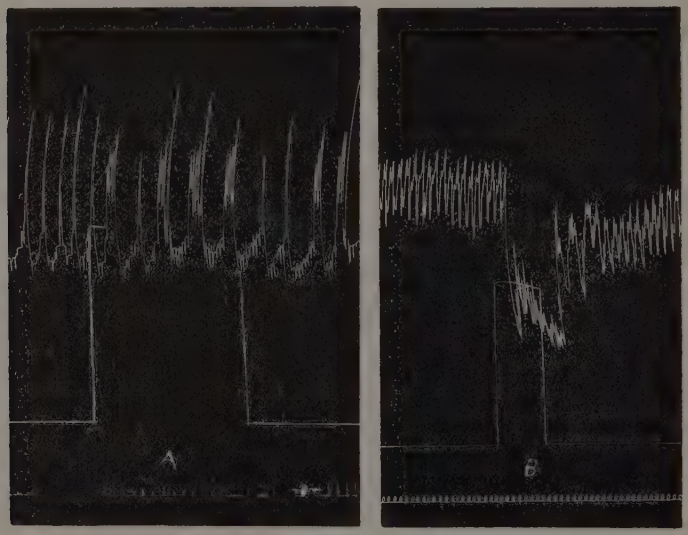

FIG. 1.

A. Control. A pressure of 68 mm. Hg. was applied to an intestinal loop for 16 seconds without any effect on the gastric contractions. B. After a previous distention of the intestinal loop for 24 hours at a pressure of 75 mm. a loop distention lasting only 8 seconds lowered the tonus of the stomach and reduced the height of contractions. The same dog was used for both records.

After the loops were distended with a pressure of 75 mm. for a period of 24 hours, the results were strikingly different. With pressures above 75 mm. there were only 4 failures in 26 tests. With pressures below 75 mm. extending as low as 25 mm., there were only 4 failures in 36 trials.

Very clearly then continued intestinal pressure lowers the

threshold for reflex gastric inhibition from intestinal distention. Whether this is an increased irritability of the gastric inhibitory mechanism or of intestinal receptors cannot be said. It is of interest to know that increased mechanical pressure in the lumen of the intestine may reinforce a visceral inhibitory reflex and especially one concerned with gastric motility.

8487 C

Liver Lipids of the White Rat Following Chloroform Poisoning, Insulin Administration and Fungus Infection.

P. L. MACLACHLAN. (Introduced by W. R. Bloor.)

From the Department of Biochemistry and Pharmacology, School of Medicine and Dentistry, The University of Rochester.

The effect of liver injury on the amount and distribution of the liver lipids has been the subject of a limited amount of investigation, with a view to gaining a better understanding of the relation between the phospholipids and the other fatty constituents of that organ. Theis[1] found for the normal liver tissue of beef, rabbits and humans that the relation between the phospholipid and neutral fat is quite constant and may be expressed as an equilibrium, 55 to 60% phospholipid $\rightleftarrows$ 45 to 40% neutral fat, an equilibrium which is readily displaced as a result of fatty degeneration, insulin administration and disease. The abnormal organs seldom showed either less or more total lipid than the normal tissue but the percentage of phospholipid was always less. Since results obtained in this laboratory indicated that the ratio of phospholipid to neutral fat for normal liver tissue is about 72% to 22%, higher than that reported by Theis, it was felt that this problem deserved further study.

The white rat was used as the experimental animal and the effect of chloroform poisoning, insulin administration and fungus infection (Aspergillus and Sporothrix) on the amount and distribution of the liver lipids was determined. The experiments were carried out on pairs of litter mates of the same sex and approximately the same weight, one serving as the control and the other as the experimental animal. The rats were killed by a blow on the back of the head and the livers removed immediately. The total fatty acid and phospholipid contents were determined by the oxidative methods of

[1] Theis, E. R., *J. Biol. Chem.*, 1928, **76**, 107; 1928, **77**, 75; 1929, **82**, 327.

TABLE I.

Distribution of Liver Lipids of Adult White Rat Following (a) Chloroform Poisoning, (b) Insulin Injection and (c) Fungus infection. (Calculated on the basis of moist weight.)

	Liver of Body Wt. %	Moisture %	Total Lipid %	Phospholipid %	Neutral Fat %	Cholesterol %	Phospholipid : Neutral Fat† %
(a) Rats given 1 to 2 hours chloroform anesthesia and killed 24 hours later. Liver showed distinct signs of fatty degeneration.							
Normal	2.98±0.36	68.4±0.4	4.08±0.56	2.97±0.25	0.91±0.36	0.22±0.03 (91±9)*	72 : 22 ± 6
Expl.	3.03±0.37	71.5±1.2	4.06±0.25	2.76±0.33	1.04±0.36	0.26±0.04 (85±13)	69 : 25 ± 7
(b) Rats given 3.6 to 50 units of insulin and killed 1 to 2 hour later (peak of reaction)							
Normal	2.66±0.24	68.7±0.5	4.66±0.36	3.06±0.18	1.34±0.26	0.25±0.03 (91±8)	66 : 28 ± 5
Expl.	2.36±0.10	69.4±0.2	4.28±0.19	3.18±0.26	0.83±0.18	0.26±0.02 (103±2)	74 : 20 ± 4
(c) Rats injected intraperitoneally with Aspergillus and Sporothrix and killed 2 weeks later. Definite signs of infection in the liver and the intestine.							
Expl.	2.75±0.22	68.6±0.9	4.45±0.20	3.30±0.10	0.90±0.10	0.25±0.01 (— —)	74 : 20 ± 2

*Free as % of total cholesterol.
†Expressed as % of total lipid.

Bloor.[2] Total and free cholesterol were determined by the method of Okey[3] as modified by Yasuda[4] by precipitation and oxidation of the cholesterol as the digitonide. All experimental values are the averages of duplicate determinations. From the values obtained by the above procedures the further distribution of the liver lipids was found by calculation.

The fatty degeneration of the liver caused by chloroform is characterized by the microscopic appearance of globules of fat in the tissue. However, the results of analysis of the livers of white rats following severe chloroform injury, Table I(a), show that the total lipid content remains essentially normal, indicating that the appearance of globules of fat in the liver is due to a rendering visible of fat already present rather than an infiltration of fat from outside depots. The ratio of phospholipid to neutral fat was also found to be within the limits of that for normal liver tissue. Administration of large doses of insulin or fungus infection (Aspergillus and Sporothrix) likewise caused no significant changes in the amount and distribution of the liver lipids, Table I (b) and (c).

These results are at variance with those reported by Theis in that (1) the proportion of total lipid present as phospholipid in normal liver tissue is considerably higher than he found and (2) no displacement of the normal phospholipid: neutral fat balance takes place in the case of the white rat as the result of chloroform poisoning, insulin administration or fungus infection.

8488 C

Reactions of Immature Rabbit Ovary to Gonadotropic Extracts.

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The reactions of the prepubertal rabbit to gonad-stimulating extracts of anterior pituitary and of pregnancy urine have been extensively studied[1-9] but apparently no ovarian responses have been

[2] Bloor, W. R., *J. Biol. Chem.*, 1928, **77**, 53.
[3] Okey, R., *J. Biol. Chem.*, 1930, **88**, 367.
[4] Yasuda, M., *J. Biol. Chem.*, 1931, **92**, 303.
[1] Siegmund, H., *Arch. f. Gynäk.*, 1930, **142**, 702.
[2] Brindeau, A., Hinglais, H., and Hinglais, M., *Compt. rend. Soc. de Biol.*, 1932, **111**, 604.

observed in this species at periods prior to the appearance of antra-bearing follicles. Since the rat[10–13] and guinea pig[14, 15] show luteinization of the theca cells in response to injections of similar extracts at an age when the follicle granulosa is still non-reactive, it was of interest to reexamine the immature rabbit with particular reference to possible reactions of an analogous nature.

White rabbits from a single stock, and representing age groups of approximately 15, 30, 45, 60 and 90 days, were used. (Table I.) Each group comprised 2 litters totalling 10 to 14 females. At the beginning of the experiments, one ovary was removed from 5 or 6 of the animals of each group to provide control material. One-half of the animals received an extract of sheep anterior pituitary, and the remaining half an oestrin-free extract of pregnancy urine. One-quarter cc. of the pituitary extract was equivalent to one luteinizing rat unit; ascending doses from 0.5 to 1.5 cc. were injected subcutaneously twice daily for 14 days. Ten γ of the pregnancy urine extract (A.P.L.) represented one luteinizing rat unit; 100 such units were given intravenously once daily for 14 days.

A few animals were killed at intervals during the injections, but the majority were killed on the 15th day. The ovaries were sectioned serially, except in a number of instances where they were stained for lipoids by frozen section technique. In addition to these, a large number of control ovaries from mature rabbits (normal, treated and hypophysectomized) was studied.

The Normal Prepubertal Ovary. Two of the animals in the 45-day age group showed the beginnings of antra formation in a few follicles, but true vesicular follicles were first observed in 4 out of

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[3] Clauberg, C., *Z. f. Gynäk.*, 1932, **16**, 964.

[4] Hertz, R., Hellbaum, A., and Hisaw, F. L., PROC. SOC. EXP. BIOL. AND MED., 1932, **30**, 41.

[5] Wolfe, J. M., and Ellison, E. T., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 600.

[6] Hertz, R., and Hisaw, F. L., *Am. J. Physiol.*, 1934, **108**, 1.

[7] Matsuura, Y., *J. Orient. Med.*, 1934, **20**, 77.

[8] Aron, M., *Compt. rend. Soc. de Biol.*, 1931, **108**, 1216.

[9] Watran, M., and Brabant, H., *Compt. rend. Soc. de Biol.*, 1931, **107**, 1418.

[10] Noguchi, *Jap. J. Med. Sci.* (Pharm. Sect.), 1931, **5**, 104.

[11] Selye, H., and Collip, J. B., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 647.

[12] Selye, H., Collip, J. B., and Thomson, D. L., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 780.

[13] Selye, H., Collip, J. B., and Thomson, D. L., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 800.

[14] Loeb, L., *Endocrinol.*, 1932, **16**, 129.

[15] King, A. G., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 1182.

TABLE I.

Ages of Litters in days	Body Wt., Gm.	Wt. of Both Ovaries, Mg.
16, 17	158- 252	16- 28
32, 33	375- 492	22- 62
44, 47	535- 790	28- 38
62, 65	741-1425	32- 74
90, 92	1200-1650	56-176

5 of the 60-day-old animals. Also noted at this age, deep in the juxta-medullary region of the stroma, were a few of the large polyhedral interstitial cells which, in massive formations, make up the bulk and yellow color of the mature rabbit ovary. At 90 days the interstitial stroma formed the major part of the ovary. Though most of the cells of this tissue were still elongated and compactly arranged, a considerable number (including the still scattered polyhedral cells) took lipoid stains at this period. Also taking lipoid stains from this time forward were occasional cells of the theca interna of the larger follicles. Neither in the prepubertal nor adult rabbit, however, was follicular atresia in our material found to be associated, except occasionally, with the formation of "theca nests" such as those described by Wilkerson,[16] and regularly seen in the rat and guinea pig.

Ovary of the Treated Prepubertal Rabbit. The youngest rabbits to respond to A.P.L. were the 45-day-old animals. Two of 7 treated animals showed several well-formed corpora lutea in each ovary. There were no signs of theca stimulation or of interstitial cell changes in these animals. The remaining 5 animals of this age showed neither granulosal nor thecal luteinization, nor were vesicular follicles present; clusters of large luteinized polyhedral cells, however, were present in the ovarian stroma. In both groups the uterus was still infantile at the end of the experiment.

In the 60-day-old A.P.L.-treated rabbits, 5 out of 6 animals showed well formed corpora lutea, together with numerous corpora hemorrhagica and cysts; these animals showed signs of recent progestational changes in the uterine mucosa. The remaining animal exhibited only marked hemorrhagic cyst formation, with very defective granulosa luteinization and a modified suboestric type of progestational uterine reaction. This type of reaction has been described by Wolfe and Ellison[5] as due to the intravenous method of administering pregnancy urine, and probably represents an overstimulation of the follicle. Only one of the 6 animals of this group

[16] Wilkerson, W. N., *Bull. Johns Hopkins Hosp.*, 1926, **38**, 339.

exhibited stromal changes. It is probably significant that in both 45- and 60-day-old rabbits, large interstitial cells appeared in numbers only when luteinization of the granulosa was defective.

At 90 days of age, the results of A.P.L. treatment approached those to be seen in similar treatment of mature females. Thus, 4 out of 5 animals showed extensive interstitial cell changes, though the granulosa luteinization varied widely in extent and character. Well formed corpora were found in 2 animals, and these showed typical progestational uteri. In the remaining 3, cystic and hemorrhagic corpora were observed, associated with modified progestational uterine reactions.[17]

First definite evidences of any effects from treatment with anterior pituitary extract were shown by 2 out of 5 of the 60-day-old rabbits. In these animals blood cyst formation and marked follicular atresia were apparent at the end of 15 days. Among 90-day-old animals similarly treated, one showed good corpora with typical uterine changes, while the remaining 4 showed extensive cyst and corpus hemorrhagicum formation with defective granulosa luteinization and greatly modified progestational uterine reactions. All 5 animals exhibited formation of large masses of luteinized polyhedral cells in the stroma.

The initial dose of pituitary extract given to the 60-day-old animals was probably subthreshold for this age, though potent in mature animals. Continued treatment with higher doses for as long as 14 days led to no eventual reactions either in follicle granulosa, theca or interstitial stroma, as is sometimes the case under similar circumstances in the rat and guinea pig. This was doubtless because of the rapid development of a refractory state.[18] The sera of these animals inhibited gonadotropic reactions when given, along with pituitary extract, to immature rats in the manner previously described.[19]

Summary. The interstitial stroma of immature and juvenile rabbits responds to the injection of gonadotropic extracts by the formation of large polyhedral cells which take lipoid stains. In healthy and mature females, these cells are constantly present in great numbers and constitute the bulk of the mature ovary. Since they have been found (unpublished experiments) to disappear fol-

[17] Leonard, S. L., and Hisaw, F. L., *Am. J. Physiol.*, 1930, **92**, 574.

[18] Hisaw, F. L., Hertz, R., Hellbaum, A., and Fevold, H. L., *Anat. Rec.*, 1933, 55 S, No. 40.

[19] Bachman, C., Collip, J. B., and Selye, H., PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 544.

lowing hypophysectomy of the rabbit, they are apparently normally dependent to a degree upon the anterior pituitary. In the young animal they may form in response to gonad-stimulating substances before the follicle granulosa is capable of responding, and alternately, may fail to appear when the granulosa shows prompt and widespread luteinization during treatment. The possible effects upon them of continued gonadotropic treatment, such as can be seen in the theca of the rat, may be obscured by the rapid development of refractory states in the rabbit. Other features of their reactions indicate that they are not strictly comparable with the luteinized theca cells of the rat and guinea pig. In very young rabbits gonadotropic extracts may produce corpora lutea without eliciting a progestational uterine reaction.

8489 P

Blood Pressure Responses to Insulin.

MICHEL PIJOAN. (Introduced by E. C. Cutler.)

From the Laboratory of Surgical Research, Harvard Medical School, and the Peter Bent Brigham Hospital, Boston.

Kugelmann[1] reported 7 cases of angina pectoris who developed an elevation in blood pressure and substernal pain during hypoglycemia induced by the intravenous administration of insulin. The attacks of substernal pain and symptoms brought about by this procedure were similar to those which occurred spontaneously in these patients under ordinary circumstances. The studies of Kugelmann suggested to us using insulin hypoglycemia as a method of determining blood pressure responses. We have studied the blood pressure and blood sugar changes in normal individuals, in patients with essential hypertension, and in cases of Addison's disease following the intravenous administration of insulin. In each case a blood sugar determination (Folin Micro-method using venous blood) was made following a 12-hour fast; 15 units of insulin (Mulford) were then given intravenously, and subsequent blood sugar determinations made every 10 minutes for 90 minutes. To circumvent the effects due to emotional disturbances from repeated vena punctures only one puncture was made and the lumen of the needle kept

[1] Kugelmann, B., *Klin. Wchnschr.*, 1933, **12**, 1488.

unobstructed by a flow of 1 cc. normal saline per minute. Blood pressure readings with the patient supine and resting were made every 10 minutes for one-half hour preceding the test and every 2 minutes following the administration of insulin.

In 25 normal individuals following insulin and at a variable time interval, but usually within the first 40 minutes, there was a drop in blood sugar to an average level of 30 mg. % without any appreciable changes in blood pressure. There then occurred a sharp rise in blood pressure averaging 40 mm. Hg., systolic, and 7 mm. Hg., diastolic, over the previous readings. With this event the blood sugar rose from 30 mg. % to 46 mg. % with a gradual return to the fasting level. In the study of 20 cases of essential hypertension, the same phenomena occurred in a more striking fashion. The initial fall in blood sugar following insulin was to an average of 40 mg. % without changes in blood pressure. The blood pressure then rose sharply above the previous readings 70 mm. Hg. systolic, and 20 mm. Hg. diastolic. In most cases there was a fall in blood pressure to below the original reading during the so-called crisis of hypertension. However, this fall lasted in most instances for not longer than a period of 2 or 3 minutes and the blood pressure would return to levels much higher than the original readings. This phenomenon was of a variable character. The blood pressures would then be sustained at higher levels for a duration which varied from 30 minutes to 2 hours. The rise of blood sugar at the point of the maximum initial increase of blood pressure was from 40 mg. % to 60 mg. %. Four patients with Addison's disease had an initial drop in blood sugar following insulin to 20 mg. %. This level of hypoglycemia lasted for 2 hours with a subsequent slow and gradual elevation in blood sugar in the next 3 hours. The blood pressures showed no changes whatsoever during this experiment. Controlled studies were carried out in diabetics who after having attained a hypoglycemic state with insulin responded in the same fashion as normal individuals. Insulin in varying amounts given intravenously and covered with dextrose failed to produce any reaction.

To test further the mechanism responsible for these phenomena controlled experiments were carried out with the cooperation of Dr. C. W. Walter in 2 normal dogs, which were subsequently adrenalectomized. In their normal state following intravenous insulin (1 unit per kilo) they showed a drop in blood sugar from 90 mg. % to 25 mg. % without changes in blood pressure. At this level there was a sharp rise in both systolic and diastolic tensions (from 125 mm. Hg. to 200 mm. Hg. systolic and from 80 mm. Hg. systolic

to 120 mm. Hg. diastolic). With this episode the blood sugars rose from 25 mg. % to 45 mg. % with a subsequent slow rise to normal. There were no changes in blood pressures in the adrenalectomized dogs. From these investigations we concluded that insulin hypoglycemia is an adequate method for studying changes in blood pressure, and that this hypoglycemic condition calls forth a secretion of adrenalin which is responsible for the sudden elevation in blood pressure and the initial rise in blood sugar. As far as adrenalin is concerned, the response of the hypertensive person is excessive, whereas the response in the patients with Addison's disease is negative.

8490 C

Diazotization of Proteins.

HARRY EAGLE. (Introduced by Stuart Mudd.)

From the Department of Bacteriology, University of Pennsylvania School of Medicine, Philadelphia.

Proteins treated with nitrous acid are said to be "diazotized" insofar as, like true diazonium compounds, they couple in alkaline reaction with substances containing an aromatic OH or NH_2 group, often forming a colored compound resembling the azo dyes. This reactivity of proteins is puzzling, since there is no known primary aromatic amine in protein which would react with HNO_2 to form a diazonium compound. Flick[1] contended that the reactivity of wool treated with HNO_2 was due to the formation of a nitroso rather than a diazonium compound. More recently, Morel and Sisley,[2] confirming previous work by Landsteiner[3] with salicylic acid, have reported that tyrosine treated with nitrous acid forms a diazonium compound which couples with naphthol to form azo dyes. They therefore ascribed the reactivity of diazotized protein to its tyrosine content. Their contention remains plausible even if tyrosine forms a nitrosophenol instead of a diazonium compound on treatment with HNO_2, as found in the case of phenol by Baeyer and

1 Flick, *Bull. Soc. Chem.*, 1899, **69**, 221. Quoted from Morel and Sisley.

2 Morel, A., and Sisley, P., *Bull. Soc. Chem. de France*, 1927, **41**, 1217; 1928, **43**, 881.

3 Landsteiner, K., *Centralbl. Physiol.*, 1895, **14**, Oct. 5.

Caro[4]; for nitroso compounds might also couple with phenols and aromatic amines.

The following data, however, indicate that tyrosine is not the only constituent of protein responsible for its diazotization. Instead, there is reason to believe that tryptophane is also responsible for the fact that protein treated with HNO_2 couples with aromatic amines and hydroxyls.

When tryptophane (or indole) was treated with nitrous acid in the cold, under the same conditions which lead to the "diazotization" of protein, a highly reactive compound was formed which gave a brilliant red color when added to α-naphthol in alkaline reaction (Table I). The HNO_2 probably reacted with the indole NH to form a nitrosamine.

TABLE I.

"Diazotization" of Tryptophane and Proteins, and Failure of Either Tyrosine or Protein Devoid of Tryptophane to Diazotize when Similarly Treated.

Substance tested	4N $NaNO_2$, cc.	N/1 HCl, cc.	Time of incubation	Max. dilution of neutralized mixture giving definite red coloration with α-naphthol
Tryptophane: 5 cc. neutral N/7 solution	.2	0.8	1 hr. room temp.	1:100
Tyrosine as above	.2	0.8	a. ½ " " " b. 1 " " " c. 12 " 2-5°C.	No color
Horse serum, 5 cc. 1:2 dilution (4% protein)	.8	4	12 " 2-5°C.	1:16
Casein: 5 cc. 4% solution	.8	4	12 " 2-5°C.	1:24
Egg albumin: 5 cc. 4% sol.	.8	4	12 " 2-5°C.	1:8
Gelatin: 5 cc. 4% sol.	.8	4	12 " 2-5°C.	Trace of color
Zein: 5 cc. 4% sol. in alcohol	.8	4	12 " 2-5°C.	No color

Moreover, tyrosine failed to develop any diazo-like reactivity on treatment with nitrous acid under the conditions here used; zein, which contains tyrosine but no tryptophane, also failed to form a diazo compound; and gelatin, deficient in both tryptophane and tyrosine, developed only a trace of diazo-like reactivity as compared with serum protein, egg albumin or casein similarly treated. The slight reactivity of "diazotized" gelatin may have been due to impurities in the gelatin.

It is therefore suggested that the reactivity of "diazotized" protein is in part, and perhaps largely, due to its tryptophane content.

[4] Baeyer, A., and Caro, H., *Ber. d. deutsch. chem. Gesellschaft.*, 1874, **7**, 967.

8491 C

Normal Heart-Weight, Body-Weight (HW/BW) Ratio in the Guinea Pig.

EDWARD J. VAN LIERE AND CLARK K. SLEETH.

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In the course of experimental work upon cardiac hypertrophy it was found necessary to obtain data upon the normal heart weight-body weight ratio in a large number of guinea pigs. Joseph[1] reported ratios of 4.22 gm. per kilo in 14 males and 3.91 gm. per kilo in 33 females. However, these animals had been used for other purposes in the laboratory, so there may be some question as to whether they were actually normal.

The animals reported in this paper were kept upon an adequate diet until a constant weight was reached. They were finally weighed, then killed by a blow upon the head. The thorax was opened and the heart removed from the pericardium. The great vessels were cut flush with the surface of the heart, and all 4 chambers were opened and washed free of blood. The excess moisture was removed by blotting the organ with filter paper, and the heart was then carefully weighed. The ratio was determined by dividing the heart weight, in grams, by the body weight, in kilograms.

The figures obtained are summarized in Table I.

TABLE I.

		——Heart Weight/Body Weight Ratio, Gm. per kilo——			
Sex	No. of Animals	Mean	Median	Mode	Standard Deviation of Mean
Male	77	3.17	3.14	3.16	0.015
Female	71	3.19	3.09	3.09	0.015

The fact that the standard deviation is the same for the 2 sexes does not confirm Joseph's[1] report of greater variability among males.

We feel that future work may be based upon these normal figures, since they withstood a rigorous statistical analysis.

[1] Joseph, D. R., *J. Exp. Med.*, 1908, **10**, 521.

8492 C

Tumor Incidence in Reciprocal F_1 Hybrid Mice — A x D High Tumor Stocks.

JOHN J. BITTNER. (Introduced by C. C. Little.)

From the Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine.

With the increase in the number of inbred stocks of mice interesting and important data may be obtained in the study of the incidence of certain types of cancer by making reciprocal crosses. Previously all reports on the development of tumors in hybrid generations have been observations secured by crossing strains of animals which differ greatly in the percentage of animals showing growths. In this paper we wish to consider the spontaneous tumor incidence in mice of the first filial generation made by mating individuals of two strains in which mammary gland tumors frequently develop.

Stocks of Mice. (1) The "D" Strain. A sub-strain of the Little dilute brown race, previously described by Murray[9] was used as one parental race. (2) The "A" Strain. The other stock of animals employed was originally descended from the Bagg albino strain. Data on the tumor incidence has been reported by Strong[13] and Bittner.[1] A report comparing the breeding behavior and tumor incidence of the D and the A stocks is in press (Bittner and Murray).[2]

Females from each strain were used in breeding the first generation hybrids. When the A stock females were mated to D males, the hybrids were termed ADF_1; when the D females were crossed to A males the resulting generation mice were called DAF_1. All the F_1 individuals were employed as breeders in transplantation studies.

The number of animals dying non-cancerous or developing tumors are grouped in monthly age periods in Table I. The proportion of the total cancerous mice recorded in bimonthly periods is represented graphically in Fig. 1.

The A stock's tumor curve has a bimodal character if considered by monthly periods. This tendency is eliminated if the observations are grouped in bimonthly classes. The mode, in the latter recording, was during the 12-13 months period. For the ADF_1

[9] Murray, W. S., *Am. J. Cancer*, 1934, **20**, 573.

[13] Strong, L. C., *J. Heredity*, 1934, **25**, 119.

[1] Bittner, J. J., *Am. J. Cancer*, 1935, **25**, 113.

[2] Bittner, J. J., and Murray, W. S., in press.

TABLE I.

Age mo.	A Stock			ADF_1 Hybrids			D Stock			DAF_1 Hybrids		
	No. Non-Tumor	No. Tumor	% Tumor	No. Non-Tumor	No. Tumor	% Tumor	No. Non-Tumor	No. Tumor	% Tumor	No. Non-Tumor	No. Tumor	% Tumor
4.5	24	2	0.7	23	0	0.0	9	0	0.0	0	0	0.0
5.5	11	1	0.4	17	1	0.3	8	0	0.0	2	0	0.0
6.5	16	7	2.6	8	7	2.2	10	2	1.9	3	0	0.0
7.5	16	13	4.8	2	13	4.1	10	0	0.0	3	1	2.2
8.5	19	14	5.2	3	22	6.9	6	2	1.9	4	1	2.2
9.5	7	35	13.0	6	23	7.2	0	2	1.9	1	1	2.2
10.5	14	31	11.5	2	34	10.6	7	6	5.6	1	1	2.2
11.5	9	31	11.5	4	43	13.4	8	9	8.4	1	2	4.3
12.5	10	28	10.4	3	37	11.6	5	7	6.5	0	4	8.7
13.5	7	36	13.4	0	33	10.3	0	14	13.1	1	1	2.2
14.5	8	18	6.7	1	34	10.6	4	18	16.8	0	8	17.4
15.5	7	19	7.1	0	29	9.1	9	12	11.2	0	7	15.2
16.5	1	16	6.0	1	16	5.0	6	12	11.2	1	3	6.5
17.5	0	7	2.6	0	11	3.4	6	6	5.6	1	2	4.3
18.5	0	5	1.9	1	9	2.8	4	8	7.5	1	2	4.3
19.5	3	1	0.4	1	4	1.3	4	5	4.7	0	5	10.9
20.5	0	3	1.1	0	1	0.3	1	3	2.8	1	3	6.5
21.5	0	0	0.0	1	2	0.6	1	0	0.0	0	2	4.3
22.5	0	1	0.4	0	0	0.0	0	0	0.0	0	1	2.2
23.5	0	1	0.4	0	0	0.0	1	1	0.9	2	1	2.2
24.5				0	0	0.0	0	0	0.0	0	0	0.0
25.5				0	1	0.3	1	0	0.0	0	1	2.2
26.5										0	0	0.0
27.5										0	1	0.0
28.5										0	0	0.0
29.5										0	1	0.0
30.5										1	0	0.0
Total	152	269		73	320		100	107		23	46	

hybrid generation the largest number of tumors was recorded during the eleventh month. In the D stock and DAF_1 generation the modes were in the fourteenth and fifteenth months respectively.

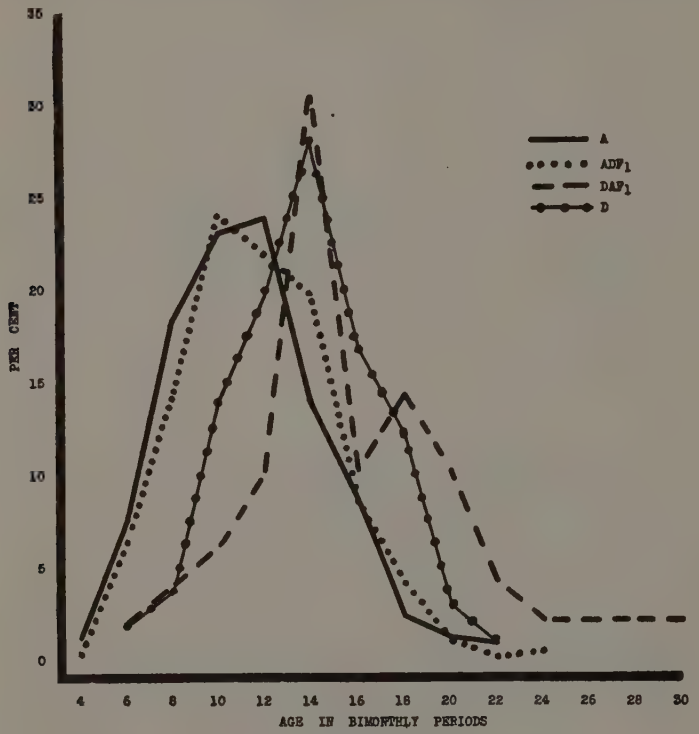

FIG. 1.
Percentage of all tumors developing in bimonthly age periods.

The mean age at observation of tumors in animals of the A and ADF_1 classes was approximately the same (12.3 and 12.1 months—Table II). Tumors of the D stock and DAF_1 generations developed at significantly later ages or 14.2 and 15.6 months respectively. The age difference between the D and DAF_1 mice is probably also of mathematical significance (1.40 ± 0.44).

The non-cancerous animals of the ADF_1 class died at an average age of 6.9 months as compared with 9.1 months for the A stock. The age at death for similar groups for the other parental strain and the DAF_1 hybrid group was considerably later, or 11 months.

More than 50% of all the animals belonging to the 2 parental

TABLE II.

Age at Observation of Tumors or at Non-cancerous Death; Means and Variations from Means for A and D Stocks and Their Reciprocal F_1 Hybrids.

Stock	No.	Mean Age mo.	Standard Deviation	Coefficient of Variation
A—Cancer	269	12.3±0.13	3.27±0.10	26.56±0.77
A—Non-Cancer	152	9.1±0.19	3.66±0.14	40.02±1.55
ADF_1—Cancer	320	12.1±0.12	3.18±0.08	26.15±0.70
ADF_1—Non-Cancer	73	6.9±0.30	3.81±0.21	55.00±3.07
D—Cancer	107	14.2±0.20	3.09±0.14	21.76±1.00
D—Non-Cancer	100	11.0±0.35	5.23±0.25	47.63±2.27
DAF_1—Cancer	46	15.6±0.39	3.91±0.28	25.04±1.76
DAF_1—Non-Cancer	23	11.8±1.00	6.81±0.68	57.81±8.49

strains which lived to be 4 months old developed mammary gland tumors (Fig. 2). For the A stock the proportion was 63.9%.

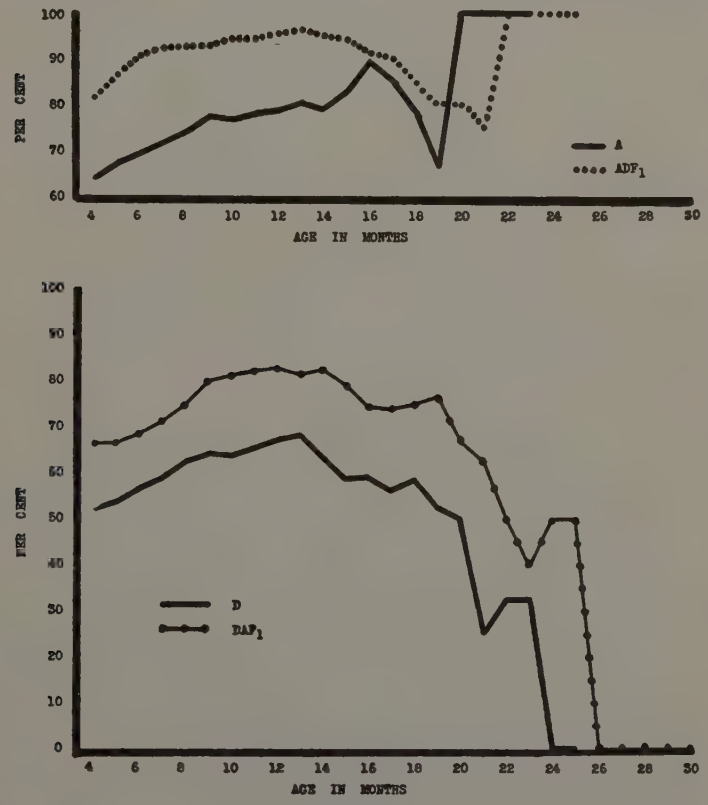

FIG. 2.

Percentage of mice living to each age period or longer which developed tumors.

This percentage increased with age until at the sixteenth month it was 89.5%. The percentage of cancerous animals in the D stock living at the beginning of the sixth month, or longer than the age of the youngest mouse to be observed with a tumor, was 56.3%. The percentage during the thirteenth month was 68.1%.

In the ADF_1 hybrid animals tumors developed in 86.5% of the animals which lived 5 months or longer. From the sixth through the seventeenth months the proportion was over 90%, reaching 96.6% at the thirteenth month. In the DAF_1 mice the first tumor was recorded during the seventh month and of the animals living, 71.9% were cancerous; by the fourteenth month, the proportion was 83.3%.

The differences between the percentages of animals to become cancerous in the various groups at the beginning of the fourth month were: A and ADF_1, $17.5\% \pm 2.1$; A and D, $12.2\% \pm 2.8$; A and DAF_1, $2.8\% \pm 4.1$; ADF_1 and D, 29.7 ± 2.6; ADF_1 and DAF_1, $14.7\% \pm 4.0$; and D and DAF_1, $15.0\% \pm 4.4$.

For the monthly periods corresponding most closely to the mean cancer ages for the strains, the percentages were: A stock, 79.0%; ADF_1 hybrids, 95.7%; D strain, 63.7%; and DAF_1 hybrids, 79.4%.

In addition to the mammary gland tumors which occurred only in females of the A stock, 4 breeding females had primary lung tumor at an average age of 20.8 months.[1] Twenty-three of 59 males of the A stock also had lung tumors. This type of cancer has never been observed in mice of either sex belonging to the D strain.

Not one of the ADF_1 hybrid females was observed which had only primary lung tumors. Two DAF_1 females were autopsied with primary lung tumors at 819 and 882 days. Two ADF_1 males, at 313 and 693 days, and one DAF_1 male, which was 775 days old when killed, also had this type of tumor. Females which had these tumors are not included in the mammary gland tumor data.

A few of the communications which have been published on the spontaneous tumor incidence observed in hybrids between high and low tumor lines are: Staff paper of the Jackson Memorial Laboratory,[12] Murray and Little,[10, 11] Korteweg[4] and Little.[5]

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12 Staff of Roscoe B. Jackson Memorial Laboratory, *Science*, 1933, **78**, 465.

10 Murray, W. S., and Little, C. C., *Genetics*, 1935, **20**, 466.

11 Murray, W. S., and Little, C. C., *Science*, 1935, **82**, 228.

4 Korteweg, R., *Nederl. Tijdschrift voor Geneeskunde*, 1934, **78**, 240.

5 Little, C. C., *J. Exp. Med.*, 1934, **59**, 229.

In the cross between the A and the D stocks, both of which were high mammary gland tumor lines, the mean tumor age in the subline of the dilute brown mice which were used was 1.9 months later than for the albino animals (7.9 x P.E.). In the F_1 animals derived from mating the A♀ by D♂ the average tumor age was approximately the same as the A stock (1.1 x P.E.) For the DAF_1 or reciprocal hybrid cross, the average age at the recording of the growths was significantly later than in the A and ADF_1 classes (8.7 and 9.2 x P.E., respectively) and possibly also when compared with the D stock (3.2 x P.E.). Thus, it will be noted that the mean tumor ages in the hybrid generation mice resembled more closely those of the female parental stock.

Tumor incidence was measured by the percentage of animals living to the beginning of each monthly age period or longer which ultimately developed mammary gland tumors. Of the animals living to the beginning of the fourth month or longer, the proportion of which developed tumors was: A, 63.9%; ADF_1, 81.4%; D, 51.7%; and DAF_1, 66.7%. Comparisons between the parental strains and the hybrids show that the difference between the A and the ADF_1 percentages was 17.5% and the D and the DAF_1 15.0%. This relationship between the maternal parental stock and their F_1 hybrid generation is maintained for several months. For the monthly age period corresponding most closely to the mean ages of tumor development according to strains, the differences between the hybrid generations and their respective maternal parental strain were the same or 16.7%.

As the number of animals developing tumors in any stock is more or less dependent upon the number of individuals living to the cancer age, the higher incidence in the hybrid generations, as compared with the parental stocks, may possibly be due to heterosis. In the DAF_1 generation the average age at death of the cancerous as well as the non-cancerous mice was much later than for the ADF_1 individuals. If longevity has any effect on tumor incidence, the DAF_1 animals should have a larger proportion of cancerous animals than the ADF_1 mice, since the average tumor age apparently would not be affected. Such was not the case. Undoubtedly of more importance was the cancer susceptibility influence transmitted by the female parent to the hybrids in determining not only the proportion of these which developed mammary gland tumors but the average cancer age as well. Thus, these data show that extra-chromosomal influences are operative in crosses of tumor by tumor strains as well as in mating of tumor by non-tumor strains.

Lynch[6, 7, 8] has demonstrated that lung tumor susceptibility may be transmitted by mating males from a high lung tumor line to females from a low tumor line. Observations on a very small number of individuals considered above and unpublished data verify these findings. They may also indicate that lung tumor susceptibility may be transmitted by either parent.

Conclusions. 1. Reciprocal crosses between 2 inbred high tumor lines indicate that: (a) The mean mammary gland tumor age in F_1 breeding females is more nearly related to that of the maternal stock. (b) The proportion of animals developing tumors in the F_1 generation was considerably greater than in the maternal stocks. (c) The relative correlation of the tumor incidence between the maternal strain and the hybrid generation was approximately the same.

2. A small number of observations show that lung tumor susceptibility may possibly be transmitted by parents of either sex from the high lung tumor race.

8493 P

Mechanism of Methylene Blue in CO-Poisoning.

MATILDA MOLDENHAUER BROOKS.

From the University of California, Berkeley.

In order to see what effect methylene blue had upon the form of the hemoglobin in rabbits poisoned with CO, spectrophotometric analyses were made on blood at regular time intervals up to 20 minutes after removal of the animal from the gas chamber. Each rabbit was allowed to remain in an atmosphere of CO plus air, (% composition not determined) until it was unconscious and barely breathing, but not long enough to cause death. CO_2 was absorbed by soda lime. The animal was then taken out, a heart puncture made and 2 drops of blood immediately placed in a small tube filled to the brim with a measured amount of 0.4% NH_4OH. This was then tightly stoppered with paraffined corks excluding air, and shaken to cause complete hemolysis. A 0.03% methylene blue solution (Merck's medicinal) dissolved in 0.9% NaCl was then in-

6 Lynch, C. J., *J. Exp. Med.*, 1924, **39**, 481.
7 Lynch, C. J., *J. Exp. Med.*, 1926, **43**, 339.
8 Lynch, C. J., *J. Exp. Med.*, 1931, **54**, 747.

jected intravenously, 1 cc. per kg., one minute after the animal had been removed from the CO chamber. Heart punctures were made 2, 4, 7, 11, 16 and 21 minutes later and samples collected as described above. Control animals were either given injections of 0.9% NaCl alone or no injections. There was no difference in the observed values between the 2 types of controls. Ten animals in each series were used. The probable error of the mean readings at each time was less than 1%. The disappearance of CO-hemoglobin and reappearance of oxyhemoglobin was then followed by spectrophotometric readings of the blood samples. The method described in previous papers[1] was used, the ratio of the extinction coefficients at 540 and 560 mμ being determined. This indicates the per cent oxyhemoglobin as compared with CO-hemoglobin present. Table I shows the progressive change in the proportion of the total hemoglobin present, the remainder in each case being CO-hemoglobin.

TABLE I.
% Oxyhemoglobin.

Time, min.	Controls	Methylene blue
0	26	26
1	43	76
3	54	96
11	63	100
21	82	100

This shows the rapid change from CO-hemoglobin to oxyhemoglobin in the case of the treated animals as compared with the slower change for the controls.

These results show definitely that methylene blue changes CO-hemoglobin into oxyhemoglobin in the blood stream and not into methemoglobin as stated by Henderson,[2] and Haggard and Greenberg.[3] Furthermore, they confirm previous findings that methylene blue is an antagonist for CO-poisoning and that it acts rapidly as opposed to the slower method advocated by Henderson.

[1] Brooks, M. M., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 1134.

[2] Henderson, Y., *Science*, 1933, **78**, 408.

[3] Haggard, H. W., and Greenberg, L. A., *J. Am. Med. Assn.*, 1933, **100**, 2001.

8494 P

Bioassay of Galactin, the Lactogenic Hormone.

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From the College of Agriculture, University of Missouri.

The increase in the crop weight of the common pigeon, while a valuable qualitative test, was found unsatisfactory in the quantitative assay of galactin due to the variability of groups of birds to the same amount of hormone. As the further purification of galactin requires a reliable bioassay, a more accurate method was sought.

Instead of weight, the minimum proliferation of the crop gland was found more satisfactory. This degree of crop growth is characterized by the presence of transverse strands or lobules of cellular development and considerable opaqueness when the crop is extended.

In the assay of the estrogenic hormone, Coward and Burn[1]

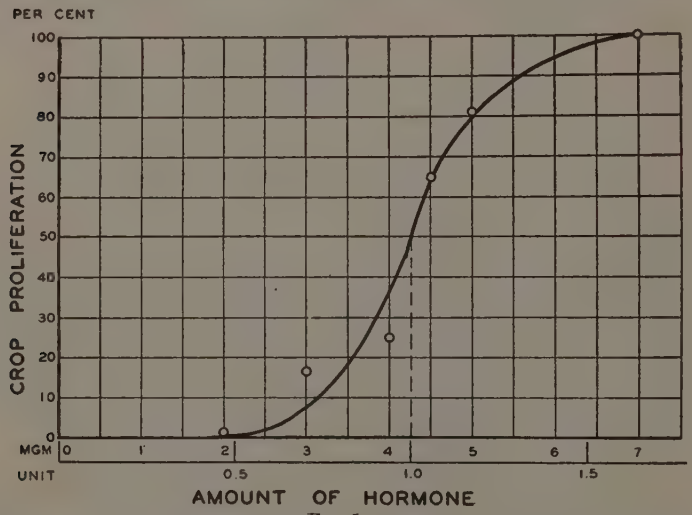

Fig. 1.

Relation between amount of hormone injected and percentage of pigeons showing crop gland proliferation. Relation is described by a characteristic sigmoid curve which indicates extreme sensitivity to small amount of hormone in region of 50% response. The pigeon unit is based upon this curve, one unit of the hormone being the amount required to give a 50% response.

*Contribution from the Department of Dairy Husbandry, Missouri Agricultural Experiment Station, Journal Series No. 437.

1 Coward, K. H., and Burn, J. H., *J. Physiol.*, 1927, **63**, 270.

made use of the variation in the response of rats as indicated by the percentage showing a positive reaction at various levels of administration. The adaptability of this method of bioassay to galactin has been studied. A characteristic sigmoid curve with maximum sensitivity in the region of 50% response was obtained (Fig. 1). An assay method has therefore been devised using minimum proliferation combined with the principle of percentage response.

The details of the assay method follow: Common pigeons weighing from 260 to 340 gm. are used. The light young birds and the very heavy birds are less uniform in their response. As no sex difference has been noted, both males and females may be used. Experience indicates that about 20 birds should be used. The extract is injected once daily for 4 days just beneath the subcutaneous tissue into the breast muscle.† The crop glands are examined on the 5th day for proliferation.

A pigeon unit of galactin is defined as the total amount of hormone injected during a period of 4 days which will cause a minimum but definite proliferation of the crop glands of $50 \pm 11\%$ of 20 common pigeons weighing 300 ± 40 gm.

The results obtained by this method using a desiccated anterior pituitary powder (Table I) indicate that the method is quite accurate in the region of 50% response.

TABLE I.

Dose of Hormone Mg.	Positive Responses %
14	79
13	100
9	41
9	38
10	53
10	53

According to Burn[2] the standard deviation of a group of 20 animals showing a 50% response is $\pm 11\%$, and 2 out of 3 assays should fall within this limit. If twice the standard deviation be taken, then 21 out of 22 assays will fall between that limit.

†If a more sensitive test is desired the injection of the extract intradermally over the crop gland as described by Lyons and Page, PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1049, may be employed.

[2] Burn, J. H., *Physiol. Rev.*, 1930, **10**, 146.

8495 C

Determination of Glucose, Galactose, and Lactose in a Mixture of the Three Sugars.

MARY SCOTT AND EDWARD S. WEST.

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Harding and coworkers[1] have devised a method of estimating a number of the common sugars when present in a mixture. They utilized *Proteus vulgaris, M. krusei, S. marxianus,* and *M. tropicalis* for the differential removal of sugars in a mixture of glucose, fructose, galactose, sucrose, maltose and lactose. This procedure combined with reduction values before and after hydrolysis provided a reasonably satisfactory method of determining the individual sugars in the mixture.

The writers have found it possible to determine glucose, galactose, and lactose in a mixture by a similar, though simplified technique which avoids the use of organisms other than baker's yeast. An improved method for the hydrolysis of lactose was devised which gives complete decomposition as compared with the 72% hydrolysis by the method of Harding and Grant.[2]

Galactose or lactose mixed with glucose were satisfactorily recovered after fermentation with washed baker's yeast. Table I shows recoveries of different quantities of glucose, galactose and lactose from solutions of all three. The data are not selected and represent all determinations made. The recoveries of galactose and lactose are not quite as good as those for glucose as might be expected due to the greater number of procedures involved in their estimation. 1. The titration values for the mixture are determined by a Shaffer-Somogyi[3] reagent (79.5 gm. Na_2CO_3, 1 gm. of KI, and 21 gm. of $NaHCO_3$ per 1) both (A) before and (B) after fermentation using 5 cc. of a 15% suspension of washed baker's yeast[4] (Fleischmann) and 14 cc. of the sugar solution made first just acid to congo with a drop of H_2SO_4.

2. The original sugar solution is mixed with an equal volume of 2 N H_2SO_4 in a 25x200 mm. tube fitted with a rubber stopper car-

1 Harding, V. J., and Nicholson, T. F., *Biochem. J.*, 1933, **27**, 1092.

2 Harding, V. J., and Grant, G. A., *J. Biol. Chem.*, 1931, **94**, 538.

3 Shaffer, P. A., and Somogyi, M., *J. Biol. Chem.*, 1933, **100**, 696.

4 Somogyi, M., *J. Biol. Chem.*, 1927, **75**, 35. Starch was removed during centrifugation.

TABLE I.
Recovery of Glucose, Galactose and Lactose from Solutions of All Three.

Mg. in 5 cc.			Recovery, %		
Glucose	Galactose	Lactose	Glucose	Galactose	Lactose
1.25	1.25	1.25	101	100	98
"	"	"	103	98	97
1.00	1.00	1.00	103	99	107
"	"	"	104	101	108
"	"	"	100	97	110
0.50	0.50	0.50	100	102	100
"	"	"	100	100	102
"	"	"	96	100	112
"	"	"	98	104	102
"	"	"	99	112	98
"	"	"	98	94	106
0.50	0.50	1.00	102	100	100
"	"	"	99	114	100
"	"	"	100	102	100
0.50	1.00	0.50	104	108	99
"	"	"	102	104	98
1.00	0.50	0.50	98	109	102
"	"	"	102	104	96
		Aver.	100.4	102.6	101.9

rying a 12 cm. capillary tube. The mixture is heated for 2.5 hours in a boiling water bath, cooled, poured into an Erlenmeyer flask and about 0.5 gm. of solid ferric sulfate per 50 cc. of solution are added.* The solution is then neutralized with precipitated $BaCO_3$ (until solution does not redden blue litmus) and thoroughly shaken. It is then filtered, the filtrate made just acid to congo with drops of concentrated H_2SO_4, and again filtered.

Titration values on the final filtrate are run (C) before and (D) after fermentation. Since the hydrolyzed solution was diluted with an equal volume of acid the titration values must be doubled for comparison with the original solution.

In determining the titration values of the above solutions which are acid (B, C, D.), 5 cc. portions are accurately pipetted into sugar tubes, 2 drops of phenol red (0.04% in water) added, followed by drops of 0.5 N NaOH to the red color of the indicator. The sugar reagent is then added and the titration value determined in the usual way.

Calculations. Curves for the sugar reagent used were prepared from known concentrations of the sugars. They were essentially straight lines from which factors for the different sugars were calculated.

The calculations are given for 5 cc. of the original solution.

*Added to remove sulphide found in practically all $BaCO_3$.

1. (A-B) x glucose factor = glucose in the original solution.
2. (C-D) x 2 x glucose factor = original glucose + glucose from the hydrolysis of lactose.
3. D x 2 x galactose factor = original galactose + galactose from lactose hydrolysis.
4. (2-1) = glucose from lactose. This value ÷ 0.53 = lactose. Glucose from lactose is also equal to galactose from lactose.
5. (3-4) = galactose in original solution.

8496 C

Rapid Method of Preparing Solutions of Gonadotropic Substance of Pregnant Mares' Blood.

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From the Research Laboratories, The Upjohn Company, Kalamazoo.

The method of purifying the gonadotropic substance in pregnant mares' blood by adsorption of the active material to specially prepared aluminum hydroxide followed by elution[1] is a difficult and time-consuming procedure. The method yields preparations directed to the requirements of clinical investigation but for studies on laboratory animals somewhat less purified solutions of the hormone are entirely satisfactory. For this purpose it would be desirable to have a rapid method of eliminating the bulk of the inert material from the serum or plasma of the mare and of concentrating the activity in a smaller volume.

Although there are many chemical and biological differences between the gonadotropic material present in pregnant mares' blood and that of the urine of pregnant women, we have found that the method of Katzman and Doisy[2] for the preparation of the gonadotropic substance of the latter by adsorption of the active material to benzoic acid may be successfully employed to effect a partial purification of the gonadotropic material in pregnant mares' blood. Solutions of the hormone prepared by this method contain much larger amounts of horse serum proteins than solutions prepared by adsorption to aluminum hydroxide.

One liter of citrated plasma from a pregnant mare[3] was diluted

1 Evans, H. M., Gustus, E. L., and Simpson, M. E., *J. Exp. Med.*, 1933, **58**, 569.
2 Katzman, P. A., and Doisy, E. A., *J. Biol. Chem.*, 1932, **98**, 745.
3 Cole, H. H., and Hart, G. H., *Am. J. Physiol.*, 1930, **93**, 57.

with 9 liters of water and acidified with 10% HCl (pH 3.0 to 2.5). When necessary the acidified solution was clarified in the Sharples supercentrifuge. After chilling, the solution was vigorously stirred while 750 ml. of an acetone solution of benzoic acid, saturated at room temperature, was slowly added, following which the suspension was vigorously stirred for half an hour. The suspended benzoic acid was allowed to settle, the supernatant decanted and the precipitate collected on a Büchner funnel. The filtrate and supernatant from the benzoic acid precipitate were combined and the process of adsorption repeated since a single adsorption rarely recovered all of the active material.

The combined benzoic acid precipitates were suspended in a small amount of water and the benzoic acid was extracted by shaking with chloroform. The amount of water used depended on the desired concentration of the final hormone solution. Frequently emulsions were encountered due to the separation of protein material. When this occurred the suspensions were separated and clarified in the supercentrifuge. After removing the dissolved chloroform from the aqueous solution by blowing through it a stream of nitrogen, the solution was buffered to pH 6.5 with phosphate buffer and preserved with a trace of toluene. When kept at 0°-5° such hormone solutions have been found undiminished in potency after several months.

Summary. The preparation of solutions of the gonadotropic hormone in pregnant mares' blood by adsorption to benzoic acid has been studied. As in the case of the gonadotropic substance of human pregnancy urine, the recovery of the active material of pregnant mares' blood by adsorption to benzoic acid is not quantitative. Satisfactory yields of the hormone are obtained, however, when the process of adsorption is repeated.

8497 C

Reaction of Anterior Pituitaries of Immature Female Rats to Injections of Various Amounts of Oestrin.*

J. M. Wolfe and C. S. Chadwick.

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It is well established that injection of oestrin in normal female rats induces changes in the anterior hypophysis comparable to those of pregnancy and that such injections in castrated female rats prevent typical castration changes. Daily injection of large amounts of oestrin into normal female rats for 10 days results in a marked but variable weight increase in the pituitaries. The basophiles are markedly degranulated and reduced in relative percentage. The eosinophiles also present loss of granules and a variable reduction in relative percentage. The chromophobes are greatly increased in relative percentage.[1] In the studies recorded below we have attempted to ascertain more exactly the morphologic and quantitative effects of large and small daily injection of oestrin for periods of 5 and 10 days on the various cell types of the anterior pituitaries of immature female rats. We wished to determine if the amounts of oestrin necessary to cause changes in one cell type would induce changes in the other types. Female rats, 21 to 23 days old were used. At autopsy, body, ovary and pituitary weights were obtained. Serial sections of all pituitaries were cut. Five representative sections from each were studied; differential cell counts were made on each; the number of mitoses were also counted.

In the first series, 9 rats received daily injection of 10 units of oestrin† for 5 days; a second group of 8 littermates received daily injection of 200 units for the same period. Eleven littermate controls were available. In the rats receiving 10 units daily the pituitaries were increased in weight to a mean of 3.5 mg.; the mean in the controls was 2.6 mg. The basophiles of the injected rats were moderately degranulated; the result was a decrease in the relative levels of the granular basophiles to a mean of 2.8% while this mean in the controls was 8.3%. A few of the eosinophiles in the

*These studies were aided by grants from the Division of Medical Sciences of the Rockefeller Foundation and the Grants-in-Aid Committee of the National Research Council.

1 Wolfe, J. M., Proc. Soc. Exp. Biol. and Med., 1935, **32**, 1192.

†Dihyroestrin (Progynon-B) was used; a portion of this was furnished gratuitously by Schering Corporation, Bloomfield, N. J.

injected rats were swollen and showed loss of granules. The relative percentages of eosinophiles were not decreased when compared with the controls. This may possibly be explained by the fact that in the injected rats the number of mitoses found in the eosinophiles was much greater than in the controls; the mean number per section in the injected rats was 14.7 while in the controls it was only 3.2. The mean number of mitoses per section in the chromophobes was also increased; in the injected rats it was 18.4 while in the controls it was 7.7. Mitoses were not observed in the basophiles. In the rats receiving daily injections of 200 units of oestrin for 5 days the pituitaries were increased to a mean weight of 3.9 mg. The degranulation of the basophiles was more marked than in the rats which received daily injections of 10 units; the relative level of the granular basophiles was reduced to a mean of 1%. Nongranular basophiles were increased inversely. Numerous eosinophiles in these glands showed loss of granules; as a whole the degree of granular loss from these cells was not much greater than in the animals which received only 10 units of oestrin daily. The relative levels of these cells were not decreased when compared with the controls. The number of mitoses in the eosinophiles was increased to a mean of 11.5, and in the chromophobes to a mean of 13.7.

In a second series, 17 rats received daily injections of 10 units of oestrin for 10 days; a second group of 27 littermate sisters received daily injection of 200 units for 10 days. Nineteen littermate controls were available. In the rats receiving 10 units daily the pituitaries were increased to a mean weight of 4.8 mg.; the mean in the controls was 3.3 mg. The basophiles exhibited marked degranulation; the mean level of the granular basophiles was reduced to 1%. The mean of the granular basophiles in the controls was 6.8%. Many eosinophiles showed loss of granules but this loss was not marked enough except in 2 rats to cause any appreciable decrease in the relative levels of these cells. The mean number of mitoses in both the chromophobes and eosinophiles per section was increased when compared with the controls. In the rats receiving 200 units daily the pituitaries were increased in weight to a mean of 5.8 mg. Degranulation of the basophiles in this group was practically complete; the mean level of the granular basophiles was only 0.3%. Granular loss was marked in the eosinophiles and furthermore the relative percentages of these cells were reduced to a mean of 28.3%, which was far below the mean level of these cells in the controls (38.7%). Many eosinophiles were swollen and showed loss of granules (the granules remaining in the cell took a pale

stain). In many the negative image of the Golgi apparatus was enlarged. The chromophobes were markedly increased in percentage. Many of these cells were enlarged; the cytoplasm was either dense blue or light blue and fragmentary. In many the negative image of the Golgi apparatus was enlarged. In this group the mean number of mitoses per section in the eosinophiles was 7.5; in the chromophobes it was 19.5. In the controls these 2 means were 6 and 9.6, respectively.

These studies indicate that oestrin exerts a definite influence on both the eosinophiles and the basophiles. Small amounts of oestrin apparently induce a very definite degranulation of the basophiles and a reduction in the relative percentages of the granular basophiles in a short time. Our observations further indicate that the non-granular basophiles eventually pass into chromophobes. Mitoses were not observed in the basophiles. In the case of the eosinophiles our data are suggestive that the situation is somewhat different. Injection of oestrin induces degranulation of the eosinophiles and these degranulated cells give rise to chromophobes. However, it seems that it requires more oestrin and a longer period of time to induce degranulation in a sufficient number of eosinophiles to cause a decrease in the relative percentages of these cells in the gland. It seems highly possible that this finding is at least partially due to the fact that injection of oestrin increases the number of mitoses in the eosinophiles, thus introducing a factor which tends to keep the level of these cells near the normal limit regardless of the fact that many of these cells are at the same time losing granules and passing into chromophobes. Since the same factor is apparently not active in the basophiles any degranulation of these cells results in an immediate reduction in the relative percentages of the granular cells.

8498 P

Influence of Allergy on Development of Early Tuberculous Lesions.

L. Dienes and T. B. Mallory.

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Quantitative considerations of the amount of lipoid in a few hundred tubercle bacilli compared to the amount of tubercular or other lipoid required to produce a noticeable foreign body reaction obviously render inadequate the present more or less generally accepted hypothesis purporting to explain the histogenesis of the tubercle. Moreover, such a theory completely fails to explain the development of closely similar, sometimes almost indistinguishable lesions in other infectious granulomata such as syphilis, typhoid, glanders, or brucellosis.

In a previous paper[1] concerned with the histologic reactions in hypersensitive states, the authors pointed out that bacterial allergy—defined as the first stage of the immune response to parenterally injected antigen, characteristic only of active as contrasted with passive immunity—develops much more rapidly than has generally been believed and determines on the part of the host a tissue reaction characterized by a marked infiltration with large mononuclear phagocytes. Attention was called to the fact that bacterial allergy is particularly well marked in that group of diseases in which focal mononuclear reactions characterize the histologic picture and the suggestion was made that the relationship between the two states might better be explained by the assumption that the allergy determined the type of histologic response than by the more conventional theory that the granulomatous response provoked the allergy. The present experiments were designed to test this hypothesis by a study of the time relationships of the development of allergy and the character of the histologic reactions.

Approximately 50 guinea pigs were infected with large doses of tubercle bacilli (5 to 20 mg.), strains of both low and high virulence being used, in order to insure the rapid and intensive development of generalized hypersensitiveness. The sites of primary inoculation were varied, 2 groups of animals being infected intratesticularly, one group intraperitoneally, and one both intratesticularly and subcutaneously. Tuberculin sensitiveness was tested with the

[1] Dienes, L., and Mallory, T. B., *Am. J. Path.*, 1932, **8**, 689.

intracutaneous injection of tuberculin and also by the injection of small doses of living tubercle bacilli into the skin. The animals were killed on successive days and the sites of infection and the skin tests were examined microscopically. In all groups the development of the lesions and the appearance of sensitivity were so uniform that a summary description is adequate.

Grossly, tuberculin sensitiveness was first noticeable in skin tests made 96 hours after infection. Microscopically, however, skin tests made 72 hours after infection showed a marked infiltration of the tissues with mononuclear cells—a finding which in a former paper[1] we have shown is not only a reliable indication of bacterial allergy but is distinctly more sensitive than gross examination.

When living tubercle bacilli were substituted for tuberculin and 24-hour-old skin lesions produced on successive days after the primary infection were examined histologically, it was found that a marked polymorphonuclear reaction was always present. When the skin injection was made simultaneously with the primary infection or followed it by only 24 hours, practically no other reacting cells were visible, but when 48 hours intervened between the injections some of the animals began to show significant mononuclear infiltration and after 72 and 96 hours this became constant and extensive.

It is realized that the rate of replacement of polymorphonuclears by mononuclear cells varies with the size of the infecting dose but since the dosages were uniform, it seemed not unreasonable that this should be interpreted as evidence of early tuberculin sensitivity—the relatively persistent antigen depot supplied by the bacilli making it evident that an earlier period than was possible with tuberculin.

The primary lesions—subcutaneous, testicular, and intraperitoneal—were closely compared with the skin tests. Within a few hours the bacteria were surrounded by polymorphonuclears, which rapidly led to abscess formation in the course of 48 hours. By 72 hours the abscess was surrounded by a well defined zone of large mononuclears which in succeeding days spread centrally and gradually replaced the great majority of the granulocytes. At the same time rapid fibroblastic proliferation at the periphery of the lesion resulted in true fibrous encapsulation.

Under these conditions of massive infection, it appears fair to conclude that bacterial allergy appears coincidentally with the histologic change from a simple pyogenic to a granulomatous response. That general sensitivity would occur so early with small localized infections is of course out of the question, but the possibility of the earlier development of sensitivity at the site of the lesion sug-

gested by Stewart[2] has recently received strong inferential support from the demonstration of localized antibody formation by McMasters and Hudack.[3]

8499 C

Influence of Ethyl Alcohol on Energy Metabolism of the Mammalian Heart.*

HOWARD C. PETERS, CHARLES E. REA AND J. W. GROSSMAN.
(Introduced by Maurice B. Visscher.)

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The use of the older methods of studying the isolated heart led to contradictory conclusions regarding the effect of alcohol on cardiac contraction. Sulzer,[1] using the Starling heart-lung preparation, at present the most sensitive and reliable method for this purpose, found no stimulating effect in any concentration and showed that concentrations commonly reached in the human blood stream in alcoholic intoxication produce dilatation without an increase in the work of the heart. We have confirmed this effect and studied the changes in energy metabolism associated with it.

We have used a modified heart-lung preparation, which has been previously described.[2] In some experiments the diastolic volume was allowed to increase when alcohol was added (Table I), while in others (Table II) the external diastolic volume was maintained constant throughout the experiment by adjustment of the venous return. In all cases the venous pressure had to be lowered in order to keep diastolic volume constant after alcohol. A piston recorder was used for ventricular volume recording and the heart was held in a glass cardiometer, following the technique of Starling and Visscher.[3]

After a control period of 15 to 30 minutes, during which the volume output and oxygen usage remained constant, the desired

2 Stewart, F. W., *Am. J. Path.*, 1925, **1**, 495.

3 McMasters, P. D., and Hudack, S. S., *J. Exp. Med.*, 1935, **61**, 783.

*Aided by grant 282 from the Committee on Scientific Research of the American Medical Association.

1 Sulzer, *Heart*, 1924, **11**, 141.

2 Peters, Rea and Visscher, PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 268.

3 Starling and Visscher, *J. Physiol.*, 1927, **62**, 243.

TABLE I.
Effect of Ethyl Alcohol on Energy Exchanges in the Heart.

Exp.	Estimated conc. of alcohol, %	Oxygen consumption cal./min.	Volume output cc./min.	Efficiency %
4/6/33	0	34.1	416	3.72
	.12	36.2	406	3.41
	.23	37.0	385	3.12
4/8/33	0	36.4	429	3.68
	.10	39.6	429	3.38
	.20	33.8	353	3.15
4/10/33	0	36.1	682	6.70
	.12	36.7	600	5.56
	.25	33.2	429	4.04
4/11/33	0	30.2	341	3.96
	.13	23.5	206	3.00
4/17/33	0	30.6	411	4.33
	.17	28.1	353	3.95
4/19/33	0	38.3	469	4.54
	.14	33.9	441	4.75
	.28	29.0	330	3.99
4/23/33	0	33.8	395	3.54
	.14	35.9	366	2.81
	.28	29.5	291	2.88
6/1/33	0	30.6	435	5.26
	.16	35.7	411	3.57
6/3/33	0	33.8	364	3.36
	.23	31.0	333	3.32
	—	34.5	333	2.98

TABLE II.
Influence of Alcohol on Energy Exchanges at Constant Diastolic Volume.

Exp.	Estimated conc. of alcohol, %	Oxygen consumption cal./min.	Volume output cc./min.	Efficiency %
6/13/33	0	30.9	750	9.04
	.31	14.19	84	2.79
6/14/33	0	28.5	600	7.13
	.16	13.3	188	4.36
6/16/33	0	27.1	545	7.21
	.25	18.8	50	.75
	—	17.5	23	.34
6/21/33	0	23.3	353	4.43
	.15	14.3	0	0
6/24/33	0	26.4	600	7.96
	.25	21.1	480	7.46

amount of alcohol was injected into the tubing between the stromuhr and the venous reservoir so as to insure thorough mixing before reaching the heart. Within 30 to 40 minutes after the injection the variables recorded had generally reached a new fairly constant level, and then in some experiments alcohol was injected again and the effect of the higher concentration determined. The concentration of alcohol injected was 20% except in Exp. 4/10/33,

in which 95% alcohol was added to the blood undiluted for purposes of comparison. The time of injection was generally 2 to 3 minutes.

The alcohol concentrations in per cent by weight were estimated from the amount injected, the volume of circulating blood, and the weight of the heart and lungs. The cardiac efficiencies are based on the external work of the heart only. A correction for the metabolism of the lungs was made in the experiments of Table I by subtracting 25% of the initial oxygen consumption values except in Exp. 4/10/33 where the output is large and a 20% correction was applied. The experiments of Table II are not corrected for lung metabolism.

When the ventricular volume was unrecorded (Table I) the oxygen consumption generally decreased or increased only slightly although the heart must have dilated. In Exp. 6/3/33 in which ventricular volume was recorded but not controlled, the oxygen consumption decreased slightly although the external diastolic ventricular volume increased 17 cc. immediately after injection and continued to increase. The efficiency changes are not large but show a tendency to a decrease.

In the experiments of Table II the oxygen consumption decreased at constant external diastolic volume. This decrease is definitely an effect of the alcohol as we have never obtained decreases this large in numerous preparations failing spontaneously.[4]

Since a decrease in oxygen consumption at constant external diastolic volume might be due to hydration of the myocardium, we determined the water content of 2 groups of ventricles (Table III) by drying samples at 105° to constant weight. One group comprised hearts sampled while working 45 minutes after injection of alcohol and 2 hours after completing the heart-lung preparation. The control group was sampled after working 2 hours. It appears that alcohol produced only very slight, if any, hydration. The effects on oxygen consumption are not explicable on the grounds of alteration in volume of muscle mass due to hydration.

TABLE III.

Water content of	
Alcohol treated hearts, %	Controls, %
79.2	79.4
79.1	79.2
78.1	
80.1	Av. 79.3
79.1	
Av. 79.1	

[4] Peters and Visscher, *Am. Heart J.*, in press.

The changes in external efficiency are the result mainly of the great decrease in external cardiac output. Since no measurements of coronary flow were made the actual muscle efficiency is not known. It would be unsafe to conclude that the muscle became very much less efficient after alcohol unless coronary blood flow was determined in cases where the external output decreased greatly. The important and unequivocal result of these studies is that the total energy output diminishes, at constant diastolic volume, under the influence of ethyl alcohol.

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8500 C

Some Effects of Pentobarbital on the Rabbit.

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It has been reported[1] that the intravenous administration of glucose to dogs under barbital anesthesia reduced the duration of the anesthesia by 50%. The original purpose of the investigation reported in this paper was to determine the effect of glucose administration during pentobarbital anesthesia.

Pentobarbital in doses of 40 mg. per kilo was administered to rabbits, intraperitoneally. At the time of maximum depression, as determined by muscular relaxation and diminished respiratory rate, 10 cc. of 50% glucose was slowly injected into the marginal ear vein. The results are shown in Table I.

TABLE I.
Duration of Depression after Pentobarbital.

	No. of obs.	Mean duration, min.	Mean dev.	Mean dev. of the mean
Normal-fed, no glucose	31	151	60	11
Normal-fed, with glucose	17	143	38	9
Starved, no glucose	22	226	52	11

It will be seen that the injection of glucose to normal-fed animals did not materially shorten the period of depression. However, fasting for 20 hours increased the duration of the period appreciably. This is in agreement with Reidel,[2] who obtained similar results on rabbits during "avertin" anesthesia. He reported that 20 cc. of

[1] Johnson, C. A., Luckhardt, A. B., and Lighthill, J. A., *J. A. M. A.*, 1930, **95**, 576.

[2] Reidel, Ilse, *Arch. f. Exp. Path. u. Pharm.*, 1930, **148**, 111.

20% glucose did not shorten the period of anesthesia, but fasting doubled it.

Although all of our animals received 40 mg. of pentobarbital per kilo, their response varied between no apparent depression and death. Approximately 110 observations were made on the blood sugar level before the injection of the pentobarbital and the same number of determinations were made at the time of maximum reaction to the anesthetic. The blood sugars were determined by the Somogyi[3] micro blood sugar method. The results are divided into 3 groups corresponding to "no depression," "slight depression" and "marked depression". The mean blood sugar levels are shown for each group in Tables II and III.

TABLE II.
Blood Sugar Levels Before Pentobarbital: Normal-fed.

No. of obs.	Mean, mg.	Mean dev.	Mean dev. of the mean	Subsequent reaction to anesthetic
17	110	10	2	No depression
19	109	12	3	Slight "
76	109	12	1	Marked "

TABLE III.
Blood Sugar Levels After Pentobarbital: Normal-fed.

Maximum reaction to anesthetic	No. of obs.	Mean, mg.	Mean dev.	Mean dev. of the mean
No depression	17	116	70	17
Slight "	19	104	10	2
Marked "	75	110	17	2

Since the mean deviations for each group, both initial and at depression, were greater than the differences between the means of these groups, it is evident that there is no correlation between the blood sugar levels and the susceptibility to the drug.

The effects of pentobarbital upon the blood sugar at the time of marked depression and at recovery are shown in Table IV.

The mean blood sugar level is not changed at the time of maximum depression. However, the blood sugar at this time did show

TABLE IV.
Pentobarbital and the Blood Sugar Level: Normal-fed.

	No. of obs.	Mean, mg.	Mean dev.	Mean dev. of mean
Initial	113	109	13	1
Anesthesia	110	110	18	2
Recovery	22	90	13	2

[3] Somogyi, M., *J. Biol. Chem.*, 1926, **70**, 599.

significantly increased variability. Approximately one-third of the observations showed a rise, one-third a fall, and one-third no change. This is reflected in the large mean deviation. In spite of the constancy of the mean sugar level during the depression, there is a very definite drop in this level at the time of recovery. This cannot be due to inanition since Scott[4] has shown that a 2-hour fast period produced in the rabbit a mean drop of only 5 mg.; 4 hours of fasting produced a drop of 10 mg. Since our recovery samples were taken on an average 2½ hours after the injection of the pentobarbital and show a mean drop of 20 mg., it is evident that the fall is due to something other than the inanition. Just what this mechanism may be we are at present unable to say.

8501 P

Inulin and its Suitability for Intravenous Administration in Man.

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After having administered dahlia inulin (lot 661-non-toxic) intravenously in large doses to dogs, and to man in 42 instances in doses ranging from 30 to 150 gm., without reaction, an unexplained transient reaction consisting of chills, fever, lumbar pain, nausea, vasomotor depression, herpes and anuria was encountered. The same reaction was encountered simultaneously in another laboratory where an independent sample of inulin was being used. The material that produced the reaction in our laboratory (lot 661-toxic) was a new shipment which had been purified separately by the manufacturer from the same batch of crude inulin as had supplied lot "661-non-toxic." At our request the manufacturer supplied a fresh, highly purified sample (lot 681) prepared from a new batch of dahlia roots, which proved to be, if anything, more toxic than "661-toxic", 1.0 gm. sufficing to produce either chill, fever, headache, nausea or lumbar pain. This reactive inulin appeared to be relatively innocuous for dogs, rabbits and guinea pigs, even when administered in very large doses. Boiling for 30 minutes in distilled water did not appreciably diminish the toxicity; partial hydrol-

[4] Scott, E. L., *Arch. Int. Med.*, 1929, **43**, 393.

ysis with dilute acetic acid (sufficient to increase the reducing power from 0.9 to 13% by weight) diminished the toxicity somewhat, and complete hydrolysis with N/10 H_2SO_4 decreased the toxicity considerably. However, a fairly typical reaction was obtained after the administration of 20 gm. that had been hydrolyzed in the latter manner. Spectroscopic examination of the ash of both preparations revealed traces of numerous metals, but showed no Pb or Si, and no significant differences between the toxic and nontoxic preparations.

Through the courtesy of Dr. Eaton M. Mackay and of the Bureau of Chemistry and Soils of the U. S. Department of Agriculture, we subsequently obtained a quantity of crude chicory inulin. This material was purified in the Department of Physiology, and with the exception noted below has proved to be innocuous to animals and to man. This material has, at the time of writing, been administered intravenously to man 28 times in doses of 30 to 40 gm. without reaction, and it produced no reaction in doses up to 80 gm. But during the purification of successive batches from the original supply a single reactive lot (lot 7—chicory) was encountered, produced apparently by having been unintentionally superheated during drying. Though the reactions induced by small doses (5 and 10 gm.) of this lot of chicory inulin are not identical in every individual tested, they consist of lumbar pain, temperature, nausea, bronchiolar spasm and headache, and strongly suggest that the fundamental trouble is the same here as in lots "661-toxic" and "681" of dahlia inulin.

Attempts have been made to demonstrate the presence of a new molecular species in both the reactive dahlia and chicory inulin by making successive extractions with water at 22°C., but the total hydrolysable reducing substance, total solids, native reducing power, speed of hydrolysis and the optical rotation of these extracts show no significant differences. Further investigation of the physical properties of these and other samples of inulin are now being carried out. On the supposition that the reactive samples of dahlia inulin owe their toxicity to having been superheated while drying, the Pfanstiehl Chemical Company has kindly cooperated by preparing fresh dahlia inulin purified with due regard to our precaution against this presumed danger. A sample of this new lot of inulin (1226, received January 22nd) has been tested by us on man. No untoward reactions have been observed in doses up to 100 gm., administered intravenously.

8502 P

Local Uterine Growth in Untreated Ovariectomized Rabbits.*

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From the Department of Physiology, Long Island College of Medicine.

Two principal conditions favor uterine growth, (1) hormonal, such as occurs in the presence of oestrone[1] and progesterone,[2] and (2) local, such as is observed in the gravid horn of a unilateral pregnancy. The growth of such a cornu is much greater than that which takes place in the non-gravid horn exposed to the identical hormonal environment.[3] The difference has been ascribed to the presence of the fetuses. Local uterine growth is also observed when, during pseudopregnancy, a uterine cornu is distended with rolled rubber dam.[4] Thus the 2 growth factors may be said to be essentially *hormonal* and *physical*. The present note is concerned with experiments in which an isolation of these factors is achieved, and uterine growth induced by physical factors alone in the absence of ovarian hormonal influence.

The procedure is as follows: mature rabbits are ovariectomized at the end of a week of pseudopregnancy, at which time the uteri of the various animals are in comparable structural states. After one week of castration paraffin pellets (m.p. 54°) of 2 sizes (⅛″ and ¼″ diameter, respectively, and ½-¾″ in length) are inserted *per vaginam* into a uterine horn and anchored there by a loose stitch at each end of the pellet. Control sections of uterine tissue are distended, excised and fixed in formol-acetic fixative solution. The pellets are left *in situ* for 2 weeks, at the end of which time the rabbits are opened, the distension site excised and fixed. Control sections are again taken for comparison with the site of chronic distension.

In each of 11 experiments it has been found that, in the absence of ovarian hormonal influences, marked uterine enlargement occurs.

*Aided by a grant from the Committee for Problems in Research of Sex, of the National Research Council.

† Member of the Department of Obstetrics and Gynecology.

1 Allen, E., *J. Am. Med. Assn.*, 1935, **104**, 1498.

2 Corner, G. W., *J. Am. Med. Assn.*, 1935, **104**, 1899.

3 Knaus, H. H., *Arch. f. Gynäk.*, **141**, 395; Hammond, J., *Trans. Dynamics of Development*, 1935, **10**, 93; Markee, J. E., and Hinsey, J. C., *Anat. Rec.*, 1934-35, **61**, 311.

4 Van Dyke, H. B., and Gustavson, R. G., *J. Pharmacol. Exp. Therap.*, 1929, **37**, 379.

The muscle cells undergo hypertrophy, connective tissue increases and dilatation of the small vessels takes place. The endometrium, after 2 weeks of chronic distension, is in a state of active proliferation but contains no secreting cells. These changes take place concurrently with some atrophy in non-distended portions of the same uterus. Thus it is evident that physical distension of the type employed here is, *per se,* a potent stimulus for local uterine growth. Details of the histological changes found in these experiments will be described elsewhere with an account of the action of oestrone and progesterone under similar conditions. A correlation of the degree of growth with the degree of distension will also be made at that time.

8503 P

Electrocardiographic Changes Following Coronary Sinus Occlusion in the Dog's Heart.*

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From the Laboratories of The Mount Sinai Hospital, New York City.

The only report available on electrocardiographic changes following experimental coronary sinus obturation is that published by Otto.[1] All of the dogs studied, however, had had section of extra-cardiac nerves.

During the course of experimental attempts to increase the blood supply to the heart[2, 3] we have studied the electrocardiographic findings in 66 dogs before, during, and at various intervals of time up to 4 weeks after partial or complete occlusion of the coronary sinus. This occlusion was produced by ligation or by the injection of escharotics into or around the mouth of the coronary sinus. Twenty-five dogs showed a completely obturated coronary sinus at autopsy, 11 of these having been produced by ligature alone. In 26 dogs the obturation was partial. In 15, the same procedures were employed but no obturation was produced. The electrocardiographic findings in these dogs therefore serve as controls.

*Aided by grants from the Lucius N. Littauer and Walter W. Naumburg Funds.

†George Blumenthal, Jr., Fellow in Pathology.

1 Otto, L. H., *Am. Heart J.,* 1928, **4**, 64.

2 Gross, Louis, and Blum, Lester, PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1578.

3 Blum, Lester, and Gross, Louis, *Am. J. Thor. Surg.,* in press.

Following obturation of the coronary sinus, the entire heart dilated, the veins became engorged and the left ventricle became cyanotic up to and slightly beyond the interventricular grooves. Occasionally, ecchymotic spots appeared over the left ventricle. The right ventricle maintained its normal color except for a strip adjacent to the interventricular grooves.

The minimal consistent electrocardiographic changes following such obturation were found to be:

1. Elevation of the RT transition.
2. Notching and downward direction of the main QRS deflection.
3. Inversion of the T-wave.
4. Temporary slowing of the heart rate.

The slowing of the heart rate lasted for a few minutes. The other changes tended to return to normal within 2 to 4 weeks. Partial heart block was noted twice. High T-waves were occasionally seen after the first week. Elevation of the RT transition was practically a constant finding. Downward direction and notching of the main QRS deflection occurred frequently. Inversion of the T-wave occurred occasionally.

When obturation of the coronary sinus was incomplete, the above mentioned electrocardiographic changes were inconstant. Electrocardiographic changes following deliberate or accidentally unsuccessful ligation of the coronary sinus were very infrequent.

It is suggested that the electrocardiographic changes (particularly the RS-T changes), following coronary sinus obturation may be due to myocardial ischemia attendant on venous congestion. Contributory factors may have been local injury (at the site of manipulation) together with change in position and rotation of the heart.

8504 C

Serum Bilirubin Content of the Blood of Rats Consuming a Ration Deficient in Inorganic Salts.*

James M. Orten† and Arthur H. Smith.

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That the extreme restriction of the inorganic salts of the diet of the albino rat produces striking changes in the composition of the blood has been repeatedly demonstrated in this laboratory.[1, 2, 3] A marked increase in the number of erythrocytes occurs, accompanied by a progressive decrease in the concentration of hemoglobin. As yet, no satisfactory explanation for the phenomenon has been obtained. The increase in erythrocytes cannot be due to a diminution in blood volume since the plasma and total blood volume remain within normal limits.[2] Nor is there any evidence of an increased rate of erythrocyte formation, inasmuch as no apparent reticulosis occurs during the period of the rapid increase in the number of erythrocytes.[3] It seemed desirable, therefore, to seek an explanation of the polycythemia in another direction, and the possibility that a decreased rate of destruction of erythrocytes leading to a "passive accumulation" of cells might be involved seemed worthy of investigation. As an index to the rate of erythrocyte destruction, the bilirubin content of the serum is usually employed since, given a normal liver and biliary system, there appears to be a direct relation between the two.[4, 5] If the increase in the number of erythrocytes in the blood of the "low-salt" rats is a result of a decreased rate of cell destruction there should be some detectable decrease in the concentration of bilirubin in the serum. If, however, cell destruction proceeds at a normal rate, a concentration of serum bilirubin equal to or perhaps slightly greater than normal is to be expected.

The same procedure was followed in this experiment as in a pre-

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†National Research Council Fellow in the Department of Physiological Chemistry, Yale University, 1933-34.

1 Smith, A. H., and Schultz, R. V., *Am. J. Physiol.*, 1930, **94**, 107.

2 Swanson, P. P., and Smith, A. H., *J. Biol. Chem.*, 1932, **98**, 479.

3 Orten, J. M., and Smith, A. H., *J. Biol. Chem.*, 1934, **105**, 181.

4 Magath, T. B., and Sheard, C., *Arch. Int. Med.*, 1927, **39**, 214.

5 Barron, E. S. G., *Medicine*, 1931, **10**, 77.

vious one.[5] Male albino rats of the Connecticut Agricultural Experiment Station strain weighing from 40 to 50 gm. at weaning (21 days of age) were placed in individual cages and fed the stock colony ration.[6] Those animals which attained a weight of 120 ± 4 gm. at 35 ± 2 days of age were divided into 2 groups: one, the control group (12 rats), received an "adequate synthetic" ration; the other (10 rats), received a diet extremely poor in inorganic salts. Both diets were fed *ad libitum*. The composition of these diets and of the vitamin supplements have been described in an earlier publication.[3] Quantitative analyses, recently reported,[7] of the amounts of the chief inorganic constituents present in both the "adequate" and "low-salt" diets, serve to emphasize the extreme degree of the limitation of inorganic elements in the latter ration.

At the end of a 10-week experimental period, the serum bilirubin content of the blood of the animals was determined by a modification[8] of the van den Bergh procedure. The body weights, hemoglobin concentrations, and erythrocyte counts of the various rats were likewise recorded at the end of the experimental period. The methods employed for each of the foregoing determinations, including slight modifications of the bilirubin procedure, have been described in detail elsewhere.[6, 9]

The averaged data obtained on the animals of the various groups, together with the actual limits of variation encountered, are given in Table I. The body weight, erythrocyte, and hemoglobin values of the control animals agree well with those of comparable rats consuming either the stock colony ration or the same synthetic ration.[6] The serum bilirubin values are likewise in close agreement with those obtained in stock rats[9] and rats given a similar synthetic ration (unpublished data). As has been observed in previous experiments,[1, 2, 3] the body weights and hemoglobin levels were decidedly less and the erythrocyte counts were greater in the "low-salt" animals than in the controls given the adequate ration. The bilirubin content of the serum of the low-salt rats, however, did not differ significantly from that of the control animals. In several instances, the serum bilirubin values of the low-salt animals tended to be somewhat greater than those of the controls. A slight increase of this type might be expected since there are more erythrocytes per

[6] Orten, J. M., and Smith, A. H., *Am. J. Physiol.*, 1934, **108**, 66.

[7] Smith, A. H., and Smith, P. K., *J. Biol. Chem.*, 1934, **107**, 681.

[8] Gibson, R. G., and Goodrich, G. E., PROC. SOC. EXP. BIOL. AND MED., 1933, **31**, 413.

[9] Orten, J. M., *Am. J. Physiol.*, 1936, **114**, 414.

unit volume in the blood of the former animals and therefore a greater opportunity exists for the fragmentation of the cells with the subsequent formation of bilirubin.

TABLE I.
Body Weight, Hemoglobin, Erythrocyte, and Serum Bilirubin Values in Control and Low-Salt Rats.*

Group	No. of Rats	Body Weight (gm.) Aver.	Body Weight (gm.) Range	Hemoglobin (gm. per 100 cc.) Aver.	Hemoglobin (gm. per 100 cc.) Range	Erythrocytes (M. per cmm.) Aver.	Erythrocytes (M. per cmm.) Range
Control	12	319	282-386	16.3	15.1-18.7	8.3	7.1- 8.9
Low-Salt	10	167	130-184	13.5	10.4-16.0	10.1	8.8-11.3

	Serum Bilirubin.					
	Total		Blank		Serum Bilirubin (mg. per 100 cc.)	
	Aver.	Range	Aver.	Range	Aver.	Range
Control	1.1	0.6-1.4	0.6	0.3-1.0	0.5	0.2-0.7
Low-Salt	1.5	1.0-2.2	0.8	0.3-1.4	0.7	0.2-1.0

*At the time these data were obtained the animals were 105±2 days of age and had been on experiment for 70 days.

The "total" bilirubin values of 4 additional low-salt rats, not included in the present report, were also determined. "Blank" readings in these cases could not be made because sufficient blood could not be obtained from the animals in question. It is of some importance, however, that the "total" bilirubin values obtained on the limited amount of serum available from these rats were practically identical with those reported in Table I.

Conclusion. The bilirubin content of the serum of rats ingesting a diet deficient in inorganic salts does not differ significantly from that of control animals fed an adequate ration. This finding indicates that "low-salt" polycythemia is not the result of a decrease in the rate of red cell destruction leading to a passive accumulation of erythrocytes.

8505 P

Revival from Insufficiency and Maintenance of Adrenalectomized Dogs with Low Serum Sodium and Chloride Levels.

W. W. Swingle, W. M. Parkins* and A. R. Taylor.

From the Biological Laboratory, Princeton University.

In a previous publication[1] attention was called to the fact that the dog prostrate from adrenal insufficiency, with greatly diminished volume of circulating fluid, low arterial pressure, marked hemoconcentration and exhibiting symptoms of severe dehydration and shock, retains large quantities of fluid apparently immobilized within the tissues. This tissue fluid was shown to be sufficient in quantity, when mobilized and shifted to the blood stream under the influence of cortical hormone, to revive the animal from collapse to the point where all symptoms disappear and activity, vigor and appetite return to normal.

The present communication is concerned with the relation of this internal fluid shift to serum sodium and chloride changes.

The dog exhibiting symptoms of adrenal insufficiency is unable to dilute its blood, *i. e.*, shift the tissue fluids to the blood stream to bolster up a failing circulation. However, within a few hours following hormone administration blood dilution occurs, the hemoconcentration decreases along with a rise in arterial pressure, all symptoms disappear and activity and vigor return. Accompanying these changes is a marked diuresis with outpouring of a large volume of urine.

We assumed on the basis of the experiments of Loeb, *et al.*,[2] and Harrop, *et al.*,[3] that the cortical hormone probably mobilized and redistributed sodium and chloride along with the tissue fluids. Investigation of this point, however, reveals that following hormone administration to dogs in severe insufficiency the shift of fluid from tissues to blood stream occurs despite no elevation of the serum sodium and chloride levels. On the contrary, these electrolytes may even decrease. The essential data obtained from a representative experiment are given in Table I. When given adequate hormone

*E. R. Squibb and Sons Fellow in Biological Sciences.

1 Swingle, W. W., Pfiffner, J. J., Vars, H. M., and Parkins, W. M., *Am. J. Physiol.*, 1934, **108**, 144.

2 Loeb, R. F., Atchley, D. W., Benedict, E. W., and Leland, J., *J. Exp. Med.*, 1933, **57**, 775.

3 Harrop, G. A., Soffer, L. J., Ellsworth, K., and Trescher, J., *J. Exp. Med.*, 1933, **58**, 17.

TABLE I.

Changes in Hemoconcentration (Dilution) in the Dog Revived from Adrenal Insufficiency and Maintained Free from Symptoms at Low Serum Sodium and Chloride Levels.

Date	Wt. Kg.*	Pulse per min.	B.P. mm. Hg.	Serum Na m-eq.	Serum Cl m-eq.	Hb. gm. /100 cc.	Hemat. vol. %	R.B.C. millions	Blood urea mg. %	Blood glucose mg. %	Remarks
11/6/35	7.6	68	102	140.8	114.2	11.5	38.2	5,29	33.3	80	Normal health. Extract discontinued
11/16/35	7.3	64	46	122.9	96.4	18.4	53.4	8,88	86.9	73	Severe insufficiency. Extract injected.†
11/20/35	6.9	128	106	120.7	93.0	14.9	44.8	6,76	26.6	78	Normal, no symptoms, active, vigorous.‡

*This animal had been bilaterally adrenalectomized 4 months previous to use in these experiments and maintained in normal health by daily injections of cortical hormone.

‡Dog given free access to water but all food withheld for 4 days. This animal was maintained free from symptoms for 14 days with low sodium and chloride levels and used in another experiment.

†Cortical hormone (3 cc. per kg.) injected intravenously. Thereafter similar amounts of hormone given daily intraperitoneally in divided doses.

such animals can be returned to normal health and vigor and maintained so for considerable periods (*e. g.*, 2 weeks) with negligible change of the serum sodium and chloride from their low pre-injection levels.† If the dog is to be maintained in normal condition at low serum sodium and chloride concentrations the animals should be allowed free access to water and fed a diet relatively free of these electrolytes.

Results similar to those described above for adrenal insufficiency have been obtained in adrenalectomized dogs which have had their sodium and chloride depleted by intraperitoneal injections of isotonic glucose according to the method of Darrow and Yannet,[4] and Gilman.[5] Using such animals it is possible by withholding hormone, to shift body fluids from the blood to the tissues, and by injection of adequate hormone to shift the fluid back to the blood stream despite the fact that throughout the experiment the serum sodium and chloride are at all times extremely low.

Summary. (1) The clinical condition of the adrenalectomized dog revived from severe insufficiency is independent of the serum sodium and chloride levels. (2) Such animals can be maintained in apparently normal condition for 2 weeks or longer with the serum sodium and chloride at the levels observed when the animal is verging on death from insufficiency. (3) The disappearance of symptoms is largely due to the effect of the cortical hormone on the mobilization and shift of tissue fluids to the blood stream. This shift occurs in the face of low serum sodium and chloride concentration. (4) The serum sodium and chloride levels remain unaffected by repeated large injections of cortical hormone if all food is withheld or a salt-free diet fed.

† Animals maintained free from symptoms, with serum sodium below 120 m-eq per liter and chloride below 95 m-eq, can be run through the complete cycle of adrenal insufficiency and recovery by withholding and injecting hormone. The electrolytes remain unchanged.

4 Darrow, D. C., and Yannet, H., *J. Clin. Invest.*, 1935, **14**, 266.

5 Gilman, A., *Am. J. Physiol.*, 1934, **108**, 662.

8506 P

Function of Pituitary Grafts in Mice.*

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It is only very recently that anterior pituitary tissue has been successfully grafted. Hohlweg and Junkmann,[1] Gardner and Hill,[2] and Haterius, Schweizer, and Charipper[3] have all obtained viable grafts with varying success. May[4] cited a slight increase in body size and a repair of the testes as evidence of function in 2 hypophysectomized rats bearing intraocular pituitary implants obtained from new-born rats.

In the mouse a genetical uniformity of host and donor is necessary for successful intra-testicular grafts of pituitary tissue (Strong[5]). Will the anterior lobe become functional when implanted in the testis where pressure is slight and vascularization of the graft normal? A large amount of data has been collected which very definitely establishes function of such grafts.

Brief notes on only 2 representative cases are here presented. Intra-testicular grafts become well established and vascularized within 3 weeks. Grafts which have been in the host animal for shorter periods have not been examined. Mouse 103 received an intra-testicular graft of a sister pituitary when 50 days old. The animal was hypophysectomized at 73 days of age and was killed 120 days later. The mouse ejaculated when killed with gas just as many normal male mice do. The seminal vesicles were of normal size and were filled with secretion. The testes were normal in size, and pituitary tissue could not be observed macroscopically in the sella. Serial sections of the sella showed a very tiny cluster

*This investigation has been supported, in part, by the Fluid Research Funds of the Yale University School of Medicine. Mice of intensively inbred strains were supplied by Dr. L. C. Strong.

†Further support has been given by the Committee for Research in Problems of Sex of the National Research Council through grants made to Professor Edgar Allen.

1 Hohlweg, W., and Junkmann, *Klin. Wchnschr.*, 1932, **11**, 321.

2 Gardner, W. U., and Hill, R. T., Proc. Soc. Exp. Biol. and Med., 1935, **32**, 1382.

3 Haterius, H. O., Schweizer, M., and Charipper, H. A., *Endocrinology*, 1935, **19**, 673.

4 May, R. M., *C. R. Soc. d. Biol.*, 1935, **12**, 867.

5 Strong, L. C., unpublished data.

of pituitary cells. The fragment, measuring not more than 1.5% of the volume of a normal mouse pituitary, consisted largely of intermediate and posterior lobe tissue. Such a small piece of pars anterior did not maintain the gonads of controls and other experimental animals. Our data show that somewhat more than 5% of the anterior lobe (exclusive of posterior and intermediate lobes) must be left in the sella in order to maintain the gonads. Such animals may be, therefore, considered as physiologically hypophysectomized as sexual activity and adrenal cortex are not maintained normally.

The tubules of the epididymis contained large numbers of sperm. The majority of the seminiferous tubules were normal in spermatic function, though possibly somewhat reduced in diameter, measuring an average of 190 micra. The tubules of an untreated hypophysectomized mouse (4 months) measure from 95-120 micra in cross section, those of a normal mouse being about 200 micra. The amount and structure of the interstitial tissue was normal. Interstitial cell function was indicated by the fact that the seminal vesicles had a normal epithelial lining and contained a large amount of secretion. The zona fasiculata of adrenal cortex had not atrophied.

Mouse 124 was hypophysectomized at 40 days of age, and 43 days later the pituitary and an ovary from a sister were grafted in the right testis. One seminal vesicle was removed at this time. The animal was killed 4½ months later and good ovarian and hypophyseal grafts recovered. The rudimentary male mammary glands had grown many branched ducts as a result of function of the ovarian graft. Many testis tubules contained large numbers of sperm. The remaining seminal vesicle contained some secretion and was not greatly different from the control specimen removed at biopsy. The adrenal cortex had not undergone atrophy. Only 2.8% of the pituitary remained in place, and here again the fragment was made up of anterior, intermediate, and posterior lobes. These 2 cases show that a grafted female pituitary is able to maintain normal testes, or to restore the degenerate testes of hypophysectomized animals. The female pituitary causes testicular interstitial tissue to function normally in some cases, and at the same time supports the growth and function of a grafted ovary.

8507 P

Agglutination and Precipitation Reactions in Rheumatoid Arthritis with Hemolytic Streptococci Groups A to G.

M. H. Dawson and Miriam Olmstead.

From the Department of Medicine, College of Physicians and Surgeons, Columbia University, and the Arthritis Clinic of the Presbyterian Hospital, New York City.

It has been shown[1-4] that, in a significant proportion of cases, rheumatoid arthritis sera agglutinate certain strains of hemolytic streptococci and precipitate group-specific fractions of these organisms. Lancefield's demonstration of distinct serological groups within the hemolytic streptococci[5, 6, 7] has made possible a more detailed study of the nature and significance of these immunological reactions. Agglutination and precipitation tests were carried out with rheumatoid arthritis and control sera using representative strains of Groups A to G as agglutinogens and group-specific extracts as precipitinogens. Similar studies were recently carried out by McEwen, Chasis and Alexander.[8]

Agglutination. Seventy-six rheumatoid arthritis sera were examined for agglutinins against Group A organisms, 60 against group B, 47 against group C, 67 against group D, 15 against group E, 15 against group F, and 14 against group G by the technic previously described.[3] The results were classified as "positive," "doubtful" or "negative". In classifying the reactions both the character of the agglutination and the titer were taken into consideration. Sera agglutinating in a titer of 1:160 or higher were considered "positive" only if they gave a strong reaction in lower dilutions. Sera agglutinating in a titer of 1:160, 1:320 or even higher were considered "doubtful" unless the reactions in the lower dilutions were definitely more marked than in the higher dilutions. Sera were classified as "negative" when agglutination occurred in dilutions of less than 1:40.

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1 Cecil, R. L., Nicholls, E. E., and Stainsby, W. J., *Am. J. Med. Sci.*, 1931, **181**, 12.

2 Dawson, M. H., Olmstead, M., and Boots, R. H., Proc. Soc. Exp. Biol. and Med., 1931, **28**, 421.

3 Dawson, M. H., Olmstead, M., and Boots, R. H., *J. Immunol.*, 1932, **23**, 187.

4 Dawson, M. H., Olmstead, M., and Jost, E. L., *J. Immunol.*, 1934, **27**, 355.

5 Lancefield, R. C., *J. Exp. Med.*, 1933, **57**, 571.

6 Lancefield, R. C., *J. Exp. Med.*, 1934, **59**, 441.

7 Lancefield, R. C., and Hare, R., *J. Exp. Med.*, 1935, **61**, 335.

8 McEwen, C., Chasis, H., and Alexander, R. C., Proc. Soc. Exp. Biol. and Med., 1935, **33**, 133.

The agglutination reactions with Group A organisms were similar to those previously reported.[3] Quite different results, however, were obtained with the other groups. In only 3 instances were definitely "positive" agglutinations observed with organisms *other than those of Group A*. In 2 of these 3 cases the serum also gave a strongly positive agglutination with group A strains. It was further observed that control sera from a variety of diseases agglutinated organisms other than those of group A to approximately the same degree as did rheumatoid arthritis sera. On the other hand, the only control sera which agglutinated group A organisms in a titer comparable with that observed in rheumatoid arthritis were those from proven, severe, hemolytic streptococcal infections. The results therefore indicate that the agglutination reaction in rheumatoid arthritis sera is one which is highly characteristic of group A hemolytic streptococci.

Precipitation. Reactions were carried out with group-specific extracts of groups A to G prepared by Lancefield's method.[5] It is well recognized that extracts prepared in this manner contain other constituents in addition to the C substance upon which group-specificity depends. In the case of group A and group B strains, however, comparatively pure C substance was made available through the kindness of Dr. Heidelberger and Mrs. Lancefield. These purified extracts were employed in a limited number of experiments. The tests were carried out in the same manner as previously described[4] except that smaller quantities of both serum (0.1 cc.) and extracts (0.2 cc. and 0.05) were employed.

Seventy-eight rheumatoid arthritis sera were tested against the crude HCl extracts of groups A and B, 74 against group C, 64 against group D, 36 against group E, 29 against group F and 33 against group G. The results with the group A extract were of the same general order as those previously reported.[4] Strong precipitins were observed only in those sera which gave a strongly positive agglutination reaction. These sera also gave a certain number of cross reactions with extracts of groups other than group A. but in general the precipitation was definitely less marked. Thus, of 8 sera giving +++ or ++++ reactions with group A, one also reacted with group B and 2 with group G. It is believed that these cross reactions can be accounted for by the presence of common antigenic constituents in the crude extracts. The observation that the sera of rabbits receiving large doses of vaccine or many series of small doses are apt to react with the non-specific protein constituents of other groups supports this conclusion.

Thirty-two control sera were tested against group A extract, 30 against group B, 24 against group C, 20 against group D, 11 against groups E, F, and G. Strong precipitation reactions were observed with group A extract in only 2 cases, one a case of hemolytic streptococcus septicemia (group A) and the other a case of nephritis. The serum from the case of hemolytic streptococcus septicemia also gave a strong precipitin reaction with the crude HCl extract of both groups B and G. This result further suggests that the extracts as prepared contained common antigenic constituents.

When purified group-specific carbohydrates of groups A and B were employed as precipitinogens strong reactions were obtained with the group A material only. It should be mentioned, however, that the purified material was prepared by different methods and the results may therefore not be strictly comparable.[6, 9]

These results in general are in accord with those of McEwen, Chasis and Alexander[8] although these workers obtained a greater number of cross reactions. Our findings indicate that such cross reactions can be accounted for by the presence of common antigenic constituents in the various groups and that both the agglutination and precipitation reactions in rheumatoid arthritis sera are characteristic for group A hemolytic streptococci.

8508 P

Antistreptolysin Titers in Rheumatoid Arthritis.

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The findings of Todd,[1] Coburn and Pauli,[2] and others indicate that the antistreptolysin test furnishes an accurate method for determining the presence of recent infection with hemolytic streptococci of group A. Coburn and Pauli showed that, following hemolytic streptococcal infections, the antistreptolysin titer of the serum rises rapidly, remains elevated for a variable number of months

[9] Heidelberger, M., personal communication.

[1] Todd, E. W., *Brit. J. Exp. Path.*, 1932, **13**, 248; *J. Path. and Bact.*, 1934, **39**, 299.

[2] Coburn, A. F., and Pauli, R., *J. Exp. Med.*, 1935, **62**, 137; *J. Clin. Invest.*, 1935, **14**, 769.

and gradually tends to return to normal values. In nearly all cases in their series there was an appreciable fall in titer within 6 months and within 2 years over half the cases had reached natural levels. Myers and Keefer[3] studied the antistreptolysin titer in 13 cases of active rheumatoid arthritis and found that, although the titers varied considerably, the average was not greater than in normal individuals. They stated that "in most of these patients the disease had existed for many months." Blair and Hallman[4] determined the antistreptolysin titer in 45 patients with rheumatoid arthritis and found it to be above the normal range (over 100 units per cc.) in one-third of the cases. In their report no reference was made to the duration of the disease at the time of the observations or to the nature of the onset.

We have determined the antistreptolysin titer in 219 cases of rheumatoid arthritis, including 22 cases of rheumatoid spondylitis (Marie-Strümpell). Preliminary observations indicated that in the great majority of well established cases the titer was within normal limits, irrespective of the degree of clinical activity present. However, when the sera of early cases with acute onset were examined quite different results were obtained. In the present report, therefore, the cases are divided into 2 groups, "early" and "late" cases. For convenience, cases of less than one year's duration are considered as "early" and those of longer duration are considered as "late". In addition, the nature of onset, whether acute, subacute or insidious, has been recorded in each instance.

(1) *Early cases* (less than one year's duration.) No. of cases examined 40. Median 125. (Table I.)

TABLE I.
Early Cases.

Titer	Onset	Titer	Onset	Titer	Onset	Titer	Onset
1666	Acute	200	Insidious	125	Subacute	83	Insidious
1000	"	166	Acute	125	Insidious	71	"
500+	Subacute	166	Subacute	125	"	62	"
500+	Insidious	166	"	125	"	50	"
500	Acute	166	Insidious	125	"	33	"
250	Subacute	166	Subacute	125	"	25	Subacute
250	Acute	142	"	100	Acute	22	"
250	Subacute	125	Acute	100	Subacute	20	Insidious
250	"	125	Subacute	83	"	16	Acute
250	"	125	"	83	Insidious	14	Insidious

(2) *Late cases* (more than one year's duration.) No. of cases examined 151. Median 55.

[3] Myers, W. K., and Keefer, C. S., *J. Clin. Invest.*, 1934, **13**, 155.
[4] Blair, J. E., and Hallman, F. A., *J. Clin. Invest.*, 1935, **14**, 505.

The great majority of "late" cases showed a normal titer, irrespective of whether the onset had been acute, subacute or insidious. However, it was occasionally observed that among the "late" cases acute exacerbations were attended by a marked rise in the antistreptolysin titer.

Of particular interest was a third group of cases in which the differentiation between rheumatoid arthritis and rheumatic fever could not be made with certainty. Many of these cases conformed to that type designated as "secondary chronic polyarthritis" in the German literature. Twenty-eight such cases were examined and the titers varied from 10,000 to 25, with a median of 166. As in typical cases, the great majority of early cases in this group showed a markedly increased titer, more particularly in those instances in which the onset occurred in an acute or subacute manner. It is of some interest that the median in this group was higher than that observed in typical cases of rheumatoid arthritis.

Controls. The antistreptolysin titers were determined on 91 controls—24 cases of osteoarthritis, 24 of non-articular rheumatism (neuritis, bursitis, myositis, etc.), 9 of gonococcal arthritis, 2 of gout and 32 miscellaneous sera. The median titer observed in this control group was 62. Titers above 125 were observed only in 5 instances.

Summary. In "early" cases of rheumatoid arthritis the antistreptolysin titer is usually increased. The increase in titer is particularly well marked in those cases in which the onset was acute or subacute.

8509 P

Determination of Cholic Acid in Bile and in Duodenal Drainage.

HENRY DOUBILET.* (Introduced by Louis Gross.)

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The Gregory-Pascoe[1] reaction was found to be specific for the determination of cholic acid. The Reinhold and Wilson[2] modification of this method was combined with the procedure of Harwood[3]

* Ralph Colp Fellow in Physiology.

1 Gregory, R., and Pascoe, T. A., *J. Biol. Chem.*, 1929, **83**, 35.

2 Reinhold, J. G., and Wilson, D. W., *J. Biol. Chem.*, 1932, **96**, 637.

3 Harwood, R. U., *J. Lab. and Clin. Med.*, 1934, **19**, 1003.

in such a fashion as to obviate both the use of the color filter and the necessity of carrying out the reaction in the presence of alcohol. In addition it was found that the large amount of deoxycholic acid present in human bile, although not taking part in the reaction, formed a precipitate which interfered markedly with the colorimetric procedure. This difficulty was overcome by the addition of alcohol after the Gregory-Pascoe reaction had been carried out to completion.

Method. 1-2 cc. of human or canine gall bladder bile, 3-5 cc. of canine fistula bile, 10-30 cc. of human fistula bile or 20-35 cc. of duodenal drainage material are the usual quantities measured out into a 50 cc. centrifuge tube. Three cc. of 2N KOH is then added and thoroughly mixed with the bile. When small quantities of concentrated bile are used, 15-20 cc. of water is added, the presence of alkali preventing the precipitation of protein. Following this, 3 cc. of 40% zinc sulphate is added drop by drop with constant stirring. When the contents are thoroughly mixed, the supernatant fluid is separated by centrifuging and the precipitate washed 3 times with 15 cc. quantities of hot water. The 4 water washings are poured into a 100 cc. volumetric flask. The precipitate is then washed 3 times with 20 cc. portions of 95% alcohol. For the first washing cold alcohol is used, and for the subsequent washings hot alcohol. The alcohol washings are all transferred to a 100 cc. beaker and dried on a water bath. The residue, taken up with 5 cc. 2N K_2CO_3, is combined with the water washings in the 100 cc. flask, which is made up to volume.

One cc. is pipetted from the volumetric flask into a test tube (18 mm. in diameter) and the procedure of Reinhold and Wilson is then followed. One cc. of 0.9% aqueous furfural solution and 6 cc. of 16N H_2SO_4 are added and the tube heated for 8 minutes at 70°C. in a water bath. After cooling for 2 minutes the resultant blue color is compared with that of the standard. In the cases of human bile, 7 cc. of 95% alcohol is added at this point to both the unknown and to the standard and thoroughly mixed before reading. The 2 standards, which are always prepared with the reading of each batch of unknown solutions, contain 0.2 and 0.4 mg. of cholic acid per cc. The standard solutions are made up as follows: 200 mg. cholic acid (Riedel-De Haen cholic acid dried at 110°C. for 24 hours to drive off the combined alcohol, present in this preparation) is weighed into a 50 cc. beaker and 4.7 cc. N/10 NaOH added. The beaker is warmed gently on a water bath to dissolve the cholic acid and then transferred to a 100 cc. volumetric flask,

which is made up to volume. To make up the standard containing 0.2 mg. per cc., 10 cc. of this solution is made up to 100 cc. For the standard containing 0.4 mg. per cc., 20 cc. of the solution is made up to 100 cc.

As a rule no more than 4 unknowns are prepared and read at one time, since the color tends to fade rapidly.

If the color of the unknown solution does not match in the colorimeter within 5 mm. of reading of one of the standards, measured portions are removed from the 100 cc. flask and suitably diluted or concentrated. Since different samples of bile vary in their content of cholic acid, this procedure gives a high degree of flexibility to the method.

Cholic acid added to bile of known bile acid composition could be recovered to within 5% of its theoretical value.

8510 P

Differential Quantitative Analysis of Bile Acids in Bile and in Duodenal Drainage.

HENRY DOUBILET.* (Introduced by Louis Gross.)

From the Laboratories of Mt. Sinai Hospital, New York.

By the combination of 3 different methods the analysis of bile for taurocholic acid, glycocholic acid, total conjugated bile acids, cholic acid, deoxycholic acid, total bile acids, and free bile acids has been achieved. It has also been possible to analyze duodenal drainage material for cholic acid, deoxycholic acid and total bile acids.

Methods. 1. The Cuny[1] modification of the Schmidt-Dart[2] method is used to determine the taurine and glycine nitrogen percentage. The first is multiplied by 33.87 and the second by 28.29 to give the bile acids conjugated with taurine and with glycine. The sum of these 2 figures gives the total bile acids that are conjugated. 2. Cholic acid is determined by a method outlined in the previous paper.[3] 3. The total bile acids are estimated by an iron precipitation method based partly on principles devised by Harwood.[4] After

* Ralph Colp Fellow in Physiology.

[1] Cuny, L., "Le dosage des sels biliares dans la bile et le liquide duodenal," Paris, 1930.

[2] Schmidt, C. L. A., and Dart, A. E., *J. Biol. Chem.*, 1920, **45**, 415.

[3] Doubilet, H., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 84.

[4] Harwood, R. U., *J. Lab. and Clin. Med.*, 1934, **19**, 1003.

the bile acids have been extracted and made up to 100 cc. in a volumetric flask and the cholic acid determined, the amount of solution in the volumetric flask, estimated to contain between 40-60 mg. of bile acids, is measured out into a 150 cc. beaker. This calculation is derived from the known amount of cholic acid present in the volumetric flask. The bile acids of dog bile may be considered to be approximately 100% cholic acid, while those of human bile can be assumed to be 50% cholic acid. Thus if the volumetric flask is found to contain 100 mg. cholic acid derived from dog bile, 50 cc. of the solution is measured in the beaker. If the extract is derived from human bile, only 25 cc. is taken.

The solution in the beaker is evaporated down to 30 cc. and 10 cc. of 8N KOH is added. The beaker is covered with a watch-glass and hydrolysis is carried out on a boiling water bath for 6 hours.

After hydrolysis is completed, 5N H_2SO_4 is added until the solution becomes acid, *i. e.*, the point at which the white cloudiness due to precipitated bile acids becomes permanent. About 10 cc. of 2N K_2CO_3 is then added to neutralize the excess H_2SO_4, and to dissolve the precipitated bile acids. The contents of the beaker are brought to dryness on a water bath. The beaker is dried for 20 minutes in an oven kept at 110°C. After trituration the contents are extracted 3 times with 50 cc. quantities of light boiling (35°C.) petrolic ether. The petrolic ether is boiled down each time to half its volume on an electric hot plate covered with asbestos. The petrolic ether is filtered through a small funnel holding No. 43 Whatman's filter paper.

The beaker is allowed to stand until the residual petrolic ether has evaporated and is then placed in the 110°C. oven for 15 minutes. Absolute alcohol (15 cc.) is then added and, after stirring, the alcohol is filtered into a 100 cc. beaker through the same funnel and filter paper that had been used previously. The contents of the beaker are extracted 3 times more with 15 cc. quantities of alcohol, brought to the boiling point on a hot plate. The alcoholic solution is evaporated to dryness on a steam bath, and the bile salts transferred with small quantities of water to a 15 cc. centrifuge tube, the total volume being about 9-10 cc. With a drop of phenol red solution as indicator, 2-3 drops of 5N H_2SO_4 are added to neutralize any K_2CO_3 that may have come through in the alcoholic filtrate. The centrifuge tube is then heated for 5 minutes in the boiling water bath to drive off CO_2 formed. Just sufficient NaOH is added to make the solution alkaline and to dissolve the precipitated bile acids. The contents, while still hot, are brought to a pH of about 6.9 by the addition of N/1 HCl drop by drop, the optimum point

being where the pink color, due to the phenol red, just disappears. The centrifuge tube is cooled under the water tap and about 4 cc. of a 4% $FeCl_3$ solution mixed with an equal amount of borax—boric acid buffer of pH 6.95, is added with a pipette. After standing for 15 minutes, the precipitated ferric bile salts are centrifuged and the supernatant fluid discarded. The precipitate is washed twice with N/2 Na_2SO_4 to remove the excess ferric chloride. About 10 cc. of 2N K_2CO_3 is then added and the centrifuge tube heated for half an hour in the steam bath. After centrifuging again, the supernatant fluid, containing the bile salts is discarded and the precipitated ferric carbonate is washed once with dilute K_2CO_3 to remove any bile salts still present. The precipitate is then dissolved in about 10 cc. N/1 HCl and after carefully adding 3 drops of superoxol the centrifuge tube is placed in the boiling water bath for a half hour. The ferric chloride solution thus formed is transferred to a 25 cc. volumetric flask, which is made up to volume with N/1 HCl. The amount of iron is then determined.

If, for example, the iron precipitated by the bile acids is found to be 100 mg. % and the cholic acid in the same sample 1%, then the iron precipitated by the deoxycholic acid present will be equivalent to 100 —45.4 = 44.6 mg. % Fe (since 1 mg. of cholic acid combines with 0.0454 mg. Fe). Since 1 mg. deoxycholic acid combines with 0.0474 mg. Fe, deoxycholic acid $= \frac{44.6}{0.0474} = 941$ mg. % — 0.941%. Adding the deoxycholic acid (0.941%) to the cholic acid (1%) gives the figure for the total bile acids (1.941%).

By this method cholic and deoxycholic acids added to bile of known bile acid composition can be recovered to within 7% of their theoretical value.

4. The free bile acids are determined by subtracting the conjugated bile acids from the total bile acids.

Some typical examples of differential bile acid analysis are shown in Table I.

TABLE I.

Source of Bile	Conjugated Bile Acids			Cholic Acid	Deoxycholic Acid	Total Bile Acids	Free Bile Acids
	with Taurine	with Glycine	Total				
Human G. B.	3.799%	5.062%	8.861%	4.255%	5.496%	9.751%	0.890%
Canine G.B.	11.109	0	11.109	10.416	3.572	13.988	2.879
Human Fistula	1.061	0.288	1.304	0.626	0.881	1.717	0.413
Duodenal Drainage "A" Bile	—	—	—	0.129	0.118	0.247	—

8511 P

Studies on Vasomotor Reflexes. Vasoconstriction from a Deep Inspiration of Air.

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The superficial and total blood flows of the forearm and hand were studied by the use of 4 different methods. Over 100 experiments were performed on 15 healthy adults, including 2 women. Each experiment was preceded by a rest of 15 to 30 minutes, and equally lengthy periods of control observation. The experiments lasted from 1 to 3 hours.

The plethysmograph indicates that a deep breath causes a fall in forearm volume of from 5 to 10 cc. The shrinkage begins near the end of inspiration, and reaches a maximum in from 15 to 30 seconds. Recovery is slower but complete.

The fall in volume may be analyzed further by the discontinuous blood flow method of Hewlett and Zwaluwenberg.[1] While the hand and part of the forearm are in the plethysmograph, the venous outflow is obstructed suddenly by abruptly raising to 70 or 80 mm. Hg. the pressure within a small cuff placed just centrad to the plethysmograph. The resultant changes in forearm volume indicate the rate of inflow of blood through the still open arteries. Although this method of measuring is discontinuous, it has the advantage of indicating the total blood flow through the forearm.

This method shows that on deep inspiration the blood flow begins to slow in mid-inspiration; reaches its maximum at the end of inspiration, and disappears rapidly. A record obtained in mid-expiration shows the flow already partly recovered. On deep inspiration, the blood flow through the forearm is stopped completely, for from 1 to 15 seconds, depending upon the rate and depth of the inspiration and the subject of the experiment. Experiments performed at every phase of the respiratory cycle indicate that the diminished flow is due to the inspiratory movement, for recovery becomes complete even when the breath is held at inspiration. Forced expiration after a normal inspiration has no vasoconstrictor effects. These results on total blood flow are supported by direct observation of the nailfold capillaries.

The flow of blood through the nailfold capillaries may be ob-

[1] Hewlett and Zwaluwenberg, *Heart*, 1909, **1**, 87.

served through the microscope. A deep breath results in slowing and complete stoppage of capillary flow, which returns to normal in a space of time analogous to that of the other methods. During a deep inspiration there is no discernible change in size and number of capillaries under observation. The blood flow impedance from a deep inspiration of air resembles that described by Wright[2] as occurring with the inhalation of cigarette smoke.

Since there is no accumulation of blood in the capillaries during the arrest of blood flow from a deep inspiration, it is assumed that the obstruction to the flow occurs in the arterial side of the capillary tufts. That this is so is shown by the following experiment: A large blood-pressure cuff is wrapped on the arm, the finger of which is being observed. Next the pressure in the cuff is raised suddenly to 250 mm. Hg., in order to obstruct the inflow with as little venous filling as possible. In from 15 to 45 seconds the blood in the capillary tufts stops flowing forward, and in most individuals soon moves backwards. A deep inspiration of air while the capillary stream is stagnant results in a forward movement; while a deep breath taken during the retrograde movement causes a slowing, stoppage, or reversal of the retrograde movement of blood.

These experiments suggest that the vasoconstriction from a deep breath occurs on the arterial side of the capillary tuft, a fact borne out also by the marked changes in temperature of the skin. They suggest also that the phenomenon is of neural or reflex origin, and is not due to mechanical shifts of blood into the pulmonary circulation, for such shifts are prevented by the circulatory occlusion induced.

Skin temperatures taken during a deep breath show a drop of from 1°C. to 3°C. Several deep breaths taken at one-minute intervals show a summation of effect, and a drop in from 1°C. to 6°C. That this can occur is denied specifically by Wright and Moffat,[3] and impliedly by Maddock and Coller.[4]

The reflex mechanism has not yet been analyzed further, but may be due to one or more of such factors as alveolar stretching; to vascular reflex from pulmonary blood pressure changes; or to the cooling effect of the inspired air.

Our thanks are due to Mr. Raymond L. Osborne for his assistance in this research.

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[2] Wright, *J. Am. Med. Assn.*, 1933, **101**, 439.

[3] Wright and Moffat, *J. Am. Med. Assn.*, 1934, **103**, 320.

[4] Maddock and Coller, PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 487.

8512 P

Hyperproteinemia with Reversal of the Albumin:Globulin Ratio in Lymphogranuloma Inguinale.

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In the course of an inquiry into the significance of the proteinuria observed in a case of lymphogranuloma inguinale, the protein content of the serum was determined. An unanticipated hyperproteinemia was found, confirmed by 2 subsequent examinations. Further investigation in 12 cases of lymphogranuloma inguinale revealed high serum protein values in all but 2 cases. The hyperproteinemia was more marked in the advanced stages of the disease (cases 1-7, Table I), particularly in females with rectal stricture, than in those patients presenting suppurating inguinal buboes or proctitis. We had no opportunity to study patients with initial lesions.

There was a relative and absolute increase in serum globulin in 11 cases, shown to be due to an increase in euglobulin in 2 cases, with reversal of the albumin:globulin ratio in 9 instances (Table I).* Serum albumin was less than 3.7% in only 2 cases. The fibrinogen content of the plasma and the non-protein nitrogen content of the serum were within normal limits. The serum calcium was 9.1, 10.2, 11.3 and 10.3 mg. % in cases 1, 4, 6, and 11 respectively. The osmotic pressure of the serum in case 1, against normal saline solution buffered at pH 7.4, was within normal limits.

The erythrocytic sedimentation rate[1, 2] was increased in all but 2 of the cases examined, but was within normal limits in defibrinated blood (5, 2, 6, 5 mm. in 1 hour in cases 8, 9, 10, and 11 respectively). The Takata-Ara test was found to be positive in the 3 cases examined. The Ray globulin flocculation test, carried out as with kala-azar serum, was negative. The urine contained traces of protein in 5 of the 10 cases examined. Precipitin tests were carried

* The occurrence of hyperproteinemia with reversal of the albumin:globulin ratio in lymphogranuloma inguinale appears not to have been recorded in the literature. Nicolau[1] noted an increase in the refractive index and in the viscosity of the serum in this disease and considered that these changes might be due to an increase in serum albumin. Our analyses fail to confirm Nicolau's inference.

[1] Nicolau, C. T., *Bull. et mém. Soc. med. hôp. Bucurest*, 1933, **13**, 45.

[2] Bloom, D., *Arch. Derm. and Syphil.*, 1933, **28**, 810.

TABLE I.

No.	Age	Sex	Race	Frei	Wassermann	Dmelcos	Serum				Fibrinogen	Erythrocytic Sedimentation Rate mm. 1st hour
							Total Protein %	Albumin %	Globulin %	A/G ratio		
1	48	♀	Negro	+	—	—	11.2					
							10.1	3.3	6.8	0.49		128
							10.7	2.9	7.8	0.37		
2	41	♀	”	+	Anti-comp.	—	9.3	3.7	5.6	0.66		79
3	50	♀	”	+	—	—	9.2	3.4	5.8	0.59		70
							8.8*				0.3	
4	48	♀	”	+	—	—	9.1	4.0	5.1	0.79		72
							—				0.4	
5	31	♀	”	+	—	—	8.7	3.7	5.0	0.76		56
							8.5*				0.4	
6	55	♂	White	+	—	—	8.6	4.0	4.6	0.87		64
7	30	♀	Negro	+	—	—	8.4*	3.8	4.3	0.83	0.3	45
8	44	♂	”	+	—	—	8.4	4.3	4.1	1.05		19
9	40	♂	White	+	—	—	8.2	3.9	4.3	0.91		20
10	28	♂	Negro	+	—	—	8.1	3.7	4.4	0.84		37
11	35	♂	White	+	—	—	7.9	4.3	3.6	1.19		87
12	35	♂	”	+	—	—	6.8	4.6	2.2	2.10		35

*Total plasma protein in oxalated blood (Some dilution due to shrinkage).

out in 1 instance (Dr. F. E. Kendall) to determine the nature of the protein excreted and revealed globulin as well as albumin. None of the 10 urines tested contained Bence Jones protein.

The cause of the hyperproteinemia described above is not clear. It is not the result of dehydration, of which there was no clinical evidence, since this would not account for the alteration in the A/G ratio. Moreover, hematocrit determination of blood cell volume, carried out in 3 instances, gave normal values. Unlike the changes in plasma proteins described in acute infections such as pneumonia,[3, 4] the fibrinogen content is not increased and the ratio fibrinogen:total plasma protein is decreased. The hyperglobulinemia observed in lymphogranuloma inguinale appears to be similar to that described in kala-azar,[5] leprosy,[6] and other chronic infections, perhaps also to that seen in neoplasia such as multiple myeloma.[7]

Hyperproteinemia is neither as constant nor as specific a manifestation of lymphogranuloma inguinale as is the Frei reaction. It is apparent, however, that the possibility of lymphogranuloma inguinale should be considered in cases presenting unexplained hyperproteinemia, particularly in negroes, and that this possibility should be ruled out by means of the Frei test. Lymphogranuloma inguinale is not uncommon in the United States; 54 cases have been seen at the Presbyterian Hospital in the past 2 years.

The hyperglobulinemia observed in lymphogranuloma inguinale may account, in part, for several interesting phenomena associated with the disease: the high sedimentation rate, often greatly in excess of the level consistent with the degree of obviously active infection; and the falsely positive and repeatedly anti-complementary Wassermann reactions reported in the literature,[8] and encountered in several of our cases.

Serum protein, non-protein nitrogen, fibrinogen and calcium were determined[9] by the macro-Kjeldahl, micro-Folin-Wu, a modified Cullen and Van Slyke and the Clark-Collip modification of the Kramer-Tisdall methods respectively. Albumin, globulin and the euglobulin fraction were determined by Howe's method. Bloods

[3] Gram, H. C., *Acta med. Scand.*, 1922, **56**, 107.

[4] Foster, D. P., *Arch. Int. Med.*, 1924, **34**, 301.

[5] Sia, R. H. P., and Wu, H., *China Med. J.*, 1921, **35**, 527.

[6] Frazier, C. N., and Wu, H., *Am. J. Trop. Med.*, 1925, **5**, 297.

[7] Perlzweig, W. A., Delrue, G., and Geschickter, C., *J. Am. Med. Assn.*, 1928, **90**, 755.

[8] Sulzberger, M. B., and Wise, F., *J. Am. Med. Assn.*, 1932, **99**, 1407.

[9] Peters, J., and Van Slyke, D. D., Quantitative Clinical Chemistry, **2**, Williams and Wilkins, Baltimore, 1931.

were collected with minimal stasis. The erythrocytic sedimentation rate was estimated by Westergren's modification.

We are indebted to Miss Ruth Jillson and Mrs. E. B. Gutman for the analyses, and to Dr. W. Curth and Dr. C. V. Burt, who kindly placed these cases at our disposal.

8513 P

Effect of Oestrus and Certain Gonadotropic Hormones on Life-Span of Adrenalectomized Animals.

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A. *Effect of oestrus on the life-span of the adrenalectomized dog not receiving adrenal cortical hormone.* Rogoff and Stewart[1] first demonstrated that the bitch, if adrenalectomized when in heat, could survive in normal health and without treatment of any kind, for as long as 65 days. This observation has been repeatedly confirmed in this laboratory by withholding hormone from the animal in heat.[2]

During the past 2 years we have studied 5 dogs in heat. The span of normal health varied, but in all cases it was greatly in excess of the longest time interval we have observed to intervene between cortical extract withdrawal and onset of adrenal insufficiency. The dogs were maintained without cortical hormone as follows: One dog 60 days; one 57 days; one 47 days; one 45 days; and one 40 days. At the end of these intervals adrenal insufficiency symptoms were evident and cortical hormone was injected. The arterial pressure, serum sodium, chloride, blood urea nitrogen, hemoglobin and glucose remained within normal limits throughout the period of good health.

Oestrus was experimentally induced in one adrenalectomized bitch by injections of extracts of menopause urine containing follicle-stimulating hormone of the anterior pituitary, followed by Follutein (pregnancy urine extract, E. R. Squibb & Sons). The animal

[1] Rogoff, J. M., and Stewart, G. N., *Am. J. Physiol.*, 1928, **86**, 20.

[2] Pfiffner, J. J., Swingle, W. W., and Vars, Harry M., *J. Biol. Chem.*, 1934, **104**, 701.

came into heat 10 days following the first injection. Cortical hormone was then withheld while the injections of gonadotropic factors were continued. The dog remained free from symptoms and in good health for 25 days.*

One adrenalectomized male dog injected daily with 50 units of menopause urine extract plus 100 units Follutein was maintained in normal health for 18 days. An ovariectomized adrenalectomized dog was injected intraperitoneally daily with 15-20 cc. of fresh Seitz filtered pregnancy urine. The animal survived in excellent health for 19 days.

B. *Experiments on adrenalectomized cats injected daily with anterior pituitary hormone.* Wilder[3] reported that the growth hormone of the anterior pituitary could be employed with advantage in the treatment of Addison's Disease as a supplement to adrenal cortical extracts. The material used by him was a growth hormone preparation made by E. R. Squibb & Sons. We investigated the effect of this material upon the life-span of the bilaterally adrenalectomized cat not receiving cortical hormone.

The animals were maintained in normal health for several weeks following adrenalectomy by adequate cortical hormone. The hormone was then withdrawn and the cats allowed to develop severe insufficiency. They were then injected with cortical hormone and restored to normal health. Growth hormone in daily doses of 1 cc. per kg. of body weight was substituted for cortical hormone. One male cat was maintained in excellent health for 150 days on anterior pituitary extract. At the end of this period the injections were stopped and the animal developed severe adrenal insufficiency 4 days later. One female cat survived 35 days, one female 28 days, 2 females 21 days, and one male 21 days.

The growth hormone preparation employed is known to contain considerable quantities of gonadotropic and thyreotropic factors. In the light of the observations on the bitch in heat, and the effect of injecting gonadotropic factors (*e. g.,* F.S.H. and Follutein) and the negative results reported with growth hormone by Evans, *et al.,*[4] on adrenalectomized rats, it seems reasonable to assume that the growth hormone *per se* is not the factor responsible for prolongation

* Normal oestrus can be induced in both adrenalectomized and immature unoperated dogs by merely injecting extract of menopause urine. The heat period is as prolonged as that characteristic of spontaneous oestrus. Follutein appears to shorten the interval.

[3] Wilder, R. M., *Proc. Staff Meetings Mayo Clinic,* 1934, **9**, 689.

[4] Evans, H. M., Pencharz, R. M., Meyer, K., and Simpson, M. E., *Memoirs of the University of California,* 1933, **41**, 347.

of life in the adrenalectomized animal. The gonadotropic principles contained in the material are probably the active agents.

8514 C

Comparison of the Corner-Allen and Clauberg Tests for Assay of Progestin.

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At the present time two methods for assaying progestin are in use, one involving the use of mature female rabbits, and the other, immature female rabbits. In connection with their work which showed that progestational proliferation is due to a specific corpus luteum hormone, Corner and Allen[1] developed a method for the standardization of corpus luteum extracts. The test devised consists of injecting the extract over a period of 5 days into a sexually mature doe castrated 18 hours after mating. A rabbit unit (Corner-Allen unit) is then defined as the minimum quantity of progestin which, divided into 5 equal daily doses, produces on the sixth day a state of the uterus equal to that of the eighth day of a normal pregnancy. A uterus in this state shows a +++ or ++++ proliferation.

Allen[2] discovered that, in the immature rabbit weighing from 575 to 1647 gm., progestational proliferation can be induced in the uterus by injections of progestin, provided the uterus is first influenced by a series of oestrin injections. Only a small percentage of the immature animals responded to the progestin treatment when it was not preceded by the oestrin injections. Clauberg[3, 4] confirmed the findings of Allen[2] on immature rabbits and established the Clauberg unit which is measured in the same way as the Corner-Allen unit except that 600 gm. rabbits are used and are primed with a total of 80 international units of oestrin, administered in 8 daily injections of 10 international units each, preceding the 5 daily injections of progestin.

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1 Corner, G. W., and Allen, W. M., *Am. J. Physiol.*, 1929, **88**, 326.

2 Allen, W. M., *Am. J. Physiol.*, 1930, **92**, 612.

3 Clauberg, C., *Zbl. f. Gynäk.*, 1930, **54**, 2757.

4 Clauberg, C., *Klin. Wschr.*, 1931, **10**, 1949.

McPhail[5] modified the Clauberg test slightly when he defined a unit, for use in his own laboratory, as that amount of progestin which injected over a period of 5 days, following the injection of 150 international units of oestrin over 6 days, produces an average proliferation of ++ in a group of immature rabbits of 750 to 950 gm. weight. A slight modification of the Corner-Allen test was used by Fevold, Hisaw and Leonard,[6] who defined a unit of progestin as that amount which, injected over a period of 4 days into an oestrous rabbit castrated previous to the first injection, produces complete proliferation on the fifth day. The Corner-Allen and Clauberg units, however, have been most widely adopted, the former being in general use among American workers, while the latter is more commonly used in the German laboratories.

Allen[2] found the immature rabbit uterus fully as sensitive as that of the mature rabbit to progestin treatment, and unpublished experiments of Corner of this laboratory and Elden of the Department of Obstetrics of this school, suggested that the Clauberg unit is equal to more than one-half but less than a whole Corner-Allen unit. Fels[7] concludes from his experiments on mixtures of Luteosteron C and D that the Clauberg unit is smaller than the Corner-Allen unit, but he does not set up an exact numerical relationship between the 2 units because of the great spread which he observes in the Clauberg test. He found that a small amount of a given solution often causes a stronger reaction than a larger amount of the solution, when injected into several immature rabbits at the same time. Although these experiments give some idea of the relationship between the two units, no direct and adequate comparison of the Clauberg and Corner-Allen units had been made prior to that reported in this paper.

A given extract, 0.10 cc. of which contained 13.2 mg. of solids, was assayed on both mature and immature rabbits according to the Corner-Allen and Clauberg methods. The extract was dissolved in benzene and prior to injection was diluted with Mazola (commercial maize oil) so that the total dose was contained in 1.0 cc. Eight mature animals were mated, castrated, injected, and autopsied according to the method described by Corner and Allen.[1] The results of this assay are recorded in Table I. Eleven immature rabbits were primed with 80 international units of oestrin in the

[5] McPhail, M. K., *J. Physiol.*, 1934, **83**, 145.

[6] Fevold, H. L., Hisaw, F. L., and Leonard, S. L., *J. Am. Chem. Soc.*, 1932, **54**, 254.

[7] Fels, E., *Zbl. f. Gynäk.*, 1935, **59**, 2420.

TABLE I

Group	Animal No.	Extract Injected	Degree of Proliferation	Aver. Proliferation for group
		cc.		
A	x-943	.20	++++	
	1003	.20	++++	4.0
B	1016	.15	++	
	1017	.15	+++	2.5
C	x-930	.10	++	
	1002	.10	++	2.0
D	1001	.05	++	
	1015	.05	+	1.5

TABLE II.

Group	Animal No.	Body Weight at Beginning of Treatment	Extract Injected	Degree of Proliferation	Aver. Proliferation for Group
		gm.	cc.		
A	1004	585	.075	+++	
	1005	515	.075	+++	
	1010	530	.075	+	2.33
B	1006	515	.050	+	
	1007	550	.050	++	
	1012	580	.050	++	
	1014	580	.050	+++	2.0
C	1008	520	.025	0	
	1009	645	.025	0	
	1011	530	.025	+++	
	1013	580	.025	0	0.75

form of oestra diol mono benzoate (Progynon-B, manufactured by the Schering Corporation) previously assayed on 5 rats, injected with the corpus luteum extract, and autopsied after the method of Clauberg.[3] The daily dose of oestrin was varied slightly in rabbits 1004, 1005, 1006, 1007, 1008, and 1009 with no noticeable effect on the subsequent growth and proliferation. The results with the immature rabbits are shown in Table II.

A Corner-Allen unit of this particular extract, based on the assay recorded in Table I, may be set at 0.15 cc. to 0.20 cc. since the proliferation averages for these 2 doses were 2.5 and 4.0 respectively. A +++ to ++++ reaction is required for the unit.

Comparison of groups B and C of Table I with groups A and B of Table II shows that doses of 0.15 cc. and 0.10 cc. of the extract produced proliferation averages of 2.5 and 2.0 respectively in the mature rabbits, whereas doses of 0.075 cc. and 0.050 cc., or exactly

one-half the above, produced the very similar proliferation averages of 2.33 and 2.0 in the immature rabbits. This comparison indicates that the Clauberg unit is equal to approximately one-half of a Corner-Allen unit.

It is evident from Table II that while the average proliferation of each group of immature animals corresponds rather well to the amount of progestin injected, the variation within each of the 3 groups is considerable (from + to +++ in groups A and B and from 0 to +++ in group C). This is in great contrast to the relatively uniform group reactions shown in Table I. It must also be noticed in Table II that in rabbit 1010 a dose of 0.075 cc. produced a reaction of only + whereas 0.025 caused a proliferation of +++ in rabbit 1011. Fels[7] reports this same sort of variation or spread and concludes from this that the Clauberg test is unreliable for measurement of potency of the corpus luteum hormone. McPhail[5] reports a similar variation within each group of 5 or 6 immature animals, but also shows that when the average proliferation of the group is taken, the proliferation indices can be plotted against the doses in a rough standardization curve. Clauberg does not give the details of his experiments, but one may conclude from the work of Fels and McPhail and the experiments reported in this paper, that assays based on proliferation in the uterus of the immature rabbit do not afford accuracy comparable to that of the Corner-Allen test unless large numbers of animals are used and group averages taken. In selecting a method of assay, this disadvantage of the immature rabbit test must be weighed against its obvious advantage in not requiring öophorectomy.

Summary. 1. The Corner-Allen and Clauberg methods for assay of progestin were compared by assaying a given extract on 8 mature and 11 immature rabbits. 2. The uterus of the immature rabbit showed much greater variation than that of the mature rabbit in its reaction to progestin. 3. The accuracy of the Corner-Allen test can be approached by the Clauberg test only if large numbers of animals are used and group averages taken. 4. A Clauberg unit, based on group averages, was found to equal approximately one-half of a Corner-Allen unit.

8515 P

Relation of Adrenal Glands to Experimental Pancreatic Diabetes.

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From the Physiological Laboratory, University of Chicago.

It was reported[1, 2] that unilateral adrenalectomy or interference with epinephrine secretion (by adrenal denervation, etc.), ameliorates diabetes in depancreatized dogs. It was stated that in animals already diabetic the adrenal operations diminished the glycosuria; also, that in dogs previously subjected to these operations, pancreatectomy either failed to cause or caused only a mild degree of glycosuria. Insulin requirement (on a constant diet) for controlling the glycosuria was reported to be decidedly less as the result of the adrenal operations which also were said to render animals more sensitive to insulin. In general, these statements agree with conclusions previously reported by others.

We find it necessary to revise the interpretations made from the earlier experiments and published in preliminary reports. More extensive investigation, under better conditions, and quantitative studies of the rate of epinephrine output (by the method employed by Stewart and Rogoff), on an adequate series of control animals, indicate that we were misled in the previous evaluation of our experimental data. Indeed, we are now convinced that satisfactory experiments lead to the conclusion that partial adrenalectomy, or operations for suppressing epinephrine secretion (adrenal denervation, with or without mechanical destruction of medulla), do not prevent or diminish the experimental diabetes produced by total pancreatectomy in dogs.

Although quantitative determinations demonstrated marked reduction or suppression of epinephrine output, in some of the preliminary experiments on depancreatized animals subjected to the adrenal operations, the assumption that this is responsible for reduced insulin requirement proved to be incorrect when a sufficient number of satisfactory control experiments became available. It is not significant that animals surviving only a short time after

* Aided by grants from The Commodore Beaumont Foundation and Mr. Max Manischewitz.

[1] Barnes, Scott, Ferrill and Rogoff, Proc. Soc. Exp. Biol. and Med., 1934, **31**, 524; *Am. J. Physiol.* (proc.), 1934, **109**, 35, 89, 95.

[2] Ferrill, Rogoff and Barnes, *Am. J. Physiol.* (proc.), 1935, **113**, 41.

operation required less insulin, sometimes little or none, since it is known that hyperglycemia and glycosuria may be low or may fail to develop if the post-operative condition of the animal is not good. Of those surviving much longer, the insulin requirement usually was decidedly less after a few weeks than shortly after pancreatectomy.

That reduction of epinephrine output is not responsible for the diminution in insulin requirement, when it occurs, is obvious from the following experimental observations: (1) under identical conditions (diet, etc.), control depancreatized dogs require the same relative amounts of insulin, at various periods of survival, as those subjected to adrenal operations in addition to pancreatectomy; (2) ordinary insulin requirement may be associated with a normal average epinephrine secretion (for animals under usual experimental conditions) or with a reduced or suppressed output of epinephrine from the adrenals; (3) low insulin requirement may be associated with either normal, reduced or suppressed epinephrine output; (4) insulin requirement may vary between relatively wide limits during the survival of depancreatized dogs, kept on a constant diet, regardless of the epinephrine output of the adrenal glands and it may vary in different animals, under the same conditions.

Finally, we have made the interesting observation that in depancreatized dogs, not subjected to interference with the adrenal glands but kept on a constant diet and insulin, sooner or later there is a definite reduction or suppression of epinephrine secretion. Whether this occurs in pancreatectomized animals that are not treated with insulin, and whether other possible factors may be responsible for the reduced or suppressed epinephrine output, are under investigation. This observation has an important clinical significance. It indicates that treatment of diabetes by X-ray irradiation or surgical denervation of the adrenals is not fundamentally sound, since it might be responsible for aggravating a pathologic condition already present.

8516 P

Soluble Specific Substance of Pneumococcus Type III Possessing Properties Distinct from SSS III.

GEORGES J. P. HORNUS* AND JOHN F. ENDERS. (Introduced by J. H. Mueller.)

From the Department of Bacteriology and Immunology, Harvard University Medical School.

It has been recently demonstrated that the soluble specific substance of Pneumococcus type I, as originally prepared by Heidelberger, Goebel and Avery, represented a form of the polysaccharide lacking the acetyl group which, when present, conferred upon the substance distinct physico-chemical and immunological properties.[1, 2] In the case of the type specific polysaccharide from Pneumococcus type III, purified according to the procedure described by the same authors, certain observations such as those of Ward[3] have led us to believe that an analogous alteration in chemical structure may have occurred during the chemical manipulations required for its purification. We have, therefore, attempted to prepare this material by a method which avoids insofar as possible the use of strong acids.

Six- to 8-day cultures of Pneumococcus type III in dextrose phosphate broth were concentrated over a boiling water bath to one-tenth of the original volume. The concentrate was precipitated several times with about 1.2 volumes of alcohol. Proteins were precipitated by careful addition of 1N acetic acid, until maximal precipitation was attained. Neutralization and reprecipitation with the acid was twice repeated.

After the last acid precipitation, the supernatant was made alkaline and precipitated with alcohol. The precipitate was dissolved in H_2O and 2 volumes of saturated ammonium sulfate added, the scanty precipitate discarded and the sulfate removed by dialysis against distilled water. The solution of polysaccharide in the dialyzing sack was precipitated with 1.2 volumes of alcohol. After resolution in H_2O and precipitation with acetone, it was washed with alcohol and ether, and dried *in vacuo*.

A pure white product was thus obtained. The substance, while exhibiting a strong reaction with the Molisch reagent, gave negative

* Fellow of the Rockefeller Foundation.

1 Avery, O. T., and Goebel, W. F., *J. Exp. Med.*, 1933, **58**, 731.

2 Enders, J. F., and Wu, C. J., *J. Exp. Med.*, 1934, **60**, 127.

3 Ward, H. K., *J. Exp. Med.*, 1932, **55**, 519.

TABLE I.

Carbohydrate used for absorption	Material used for testing supernatant of absorbed serum	Antipneumococcus type III horse serum absorbed with dilutions of carbohydrates					
		1/500	1/2,000	1/8,000	1/20,000	1/40,000	1/100,000
Carbohydrate H *	Carbohydrate H	—	—	—	—	±	++
SSS III	" "	++	+±	++	+++	+++	+++
Carbohydrate H	SSS III	—	—	—	—	±	++
SSS III	" "	—	—	—	±	++	+++
Carbohydrate H	Unabsorbed type III antiserum	+++	+++	++±	++	±	—
SSS III	Unabsorbed type III antiserum	++++	+++±	+++±	++	+±	+

*The preparation of specific substance described in this paper is thus designated for purposes of convenience in the tabulation of results.

biuret and Millon tests. The material precipitated only with antipneumococcus type III serum.

Like SSS III prepared according to the procedure of Heidelberger and his associates[4] the substance is precipitated from solution by $CuSO_4$, $BaCl_2$ and acetic acid. With all these reagents, SSS III gives a finely divided precipitate with persistent turbidity of the supernatant, while our substance forms large, heavy floccules, leaving at once a water clear supernatant. In a series of buffer solutions of varying pH, SSS III flocculates at pH 1.02 to pH 1.42, whereas at these hydrogen ion concentrations the new product reveals only a slight opalescence. After 3 days in these buffers it, too, exhibited a slight flocculation. This may well indicate a chemical change brought about by the high hydrogen ion concentration, leading to the formation of a substance similar, if not identical, with SSS III.

Our product contains 0.30% nitrogen. In this respect it resembles the material described recently by Heidelberger, Kendall and Scherp,[5] while the present work was in progress, but it apparently differs in its serological behavior, although this may not indicate a fundamental difference. Heidelberger's recent preparations, although precipitating more protein from antipneumococcus type III rabbit serum, nevertheless throws down about the same quantity as SSS III from antipneumococcus type III horse serum. The product which we have obtained, however, behaves differently from these preparations and from SSS III in its capacity to unite with homologous antibody in antipneumococcus horse serum. Thus, if such a serum be absorbed with a quantity of SSS III sufficient to remove all homologous antibody and appear in excess in the supernatant after removal of the precipitate, the supernatant, when brought in contact with the new product, will again yield a precipitate. The results of an experiment demonstrating this fact are recorded in Table I. The procedure was as follows: to each of one series of tubes containing 0.2 cc. of antipneumococcus type III horse serum was added 0.1 cc. of falling dilutions of SSS III and to another series, 0.1 cc. of dilutions of the new material. After thorough mixture, an overnight period at about 20°C. and centrifugation, portions of the supernatant from each were tested (ring test) with 1:1,000 dilution of both carbohydrates and with unabsorbed anti-serum to reveal any excess of the carbohydrates in the supernatant absorbed serum.

[4] Heidelberger, M., and Avery, O. T., *J. Exp. Med.*, 1924, **40**, 301.

[5] Heidelberger, M., Kendall, F. E., and Scherp, H. W., PROC. SOC. EXP. BIOL. AND MED., 1935, **33**, 188.

Certain other immunological properties of the material are now being studied.

8517 C

Grouping of Hemolytic Streptococci Isolated in Puerto Rico.*

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While studying the biological properties of hemolytic streptococci isolated from different sources in this Island, we grouped 46 strains using the precipitin reaction described by Lancefield.[1]

All extracts were first tested against group A serum. Those that gave a positive test were not tried against the other sera because cross reactions have never been observed with particular A antiserum.[2] Those that gave a doubtful or a negative result were tested in the same B, C, D, E, F, G and H antisera.

Out of 11 strains from diseased tonsils, 7 belonged to group A, 2 to group C and 2 remained unclassified. All the strains isolated from chronic discharging lesions of the face, from abscesses, from otitis media, from osteomyelitis and from the throat of patients with a diagnosis of scarlet fever were group A. Strains M_6 and M_7, isolated from the throat of cases of agranulocytic angina and rheumatoid arthritis respectively, remained unclassified, but they were not group A.

Of 15 strains from cases of recurrent lymphangitis, 14 were group A and one, group G. Strain D_1, from eczematoid dermatitis, belonged to Group C. Of 2 strains from septicemia, one was group A and the other, G.

Of the 46 strains tested, 37 were group A, 3, group C, 2, group G, and 4 remained unclassified.

* Group sera (A, B, D, E, F, G, H) were kindly sent to us by Dr. R. C. Lancefield.

1 Lancefield, R. C., *J. Exp. Med.*, 1933, **57**, 571.

2 Lancefield, R. C., personal communication.

8518 P

Carbohydrate Oxidation in Hypophysectomized Rats.

ROBERT E. FISHER AND RICHARD I. PENCHARZ. (Introduced by C. F. Cori.)

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It was noted that rats hypophysectomized* for 6 to 8 weeks, with metabolic rates 70% of those of normal controls (as determined gravimetrically by the Haldane method), frequently showed high respiratory quotients (R.Q.) after a fasting period of 24 hours. Values as high as 0.78 to 0.81 were sometimes observed, the average of a series of 19 observations being 0.75, while the average of 19 controls was 0.72. If the hypophysectomized rats were fasted longer than 30 hours, the R.Q. usually dropped to 0.73, though in a few instances it remained high for nearly 40 hours. Changes in the acid-base equilibrium were apparently not responsible for the high R.Q., as shown by determinations of the CO_2 content of the blood. Glycogen determinations after a 24-hour fast showed that the hypophysectomized animals contained considerably less muscle and liver glycogen than did normal controls. Hypoglycemia was always present, the blood sugar being around 50 mg. %.

These findings suggested that the regulatory mechanism which leads to the preservation of glycogen during fasting is disturbed after hypophysectomy. The low glycogen reserves and the tendency to hypoglycemia in hypophysectomized animals might be due, among other factors, to the removal of an inhibitory influence on carbohydrate oxidation. In order to test this assumption, a standard amount of glucose (500 mg. per 100 gm. of body weight) was fed to normal and hypophysectomized rats after a fasting period of 24 hours, the aim being to determine (a) how much glucose was oxidized in the first few hours during the period of active absorption and (b) what length of time was required for the complete disposal of the ingested sugar by oxidation.

In the first 4 hours the normal animals oxidized an average of 192 and the hypophysectomized animals an average of 160 mg. of glucose per 100 gm. of body weight.† The glucose fed was dis-

* The completeness of the operation was checked in each case at autopsy.

† Attention is called to the fact that hypophysectomized rats show a diminished rate of intestinal absorption of glucose, as Phillips and Robb (*Am. J. Physiol.*, 1934, **109**, 82) have shown and as we are able to confirm.

posed of by oxidation in about the same length of time (21.7 hours in the normal and 20 hours in the hypophysectomized animals), but since the latter had a 29% lower O_2 consumption than the controls, they derived a correspondingly larger proportion of their energy from carbohydrate oxidation. The hypophysectomized rats excreted less urinary nitrogen than the controls but slightly more than rats thyroidectomized for 4 weeks. The animals were on a high carbohydrate diet prior to the experiments.

In another series of observations normal and hypophysectomized rats were kept for 2 days on a high fat (butter) diet prior to the glucose test meal. Fat feeding depressed carbohydrate oxidation in the normal but had little effect in the hypophysectomized animals; the former oxidized an average of 144 and the latter of 190 mg. per 100 gm. of body weight per 4 hours.

Hypophysectomized rats are about 8 times more sensitive to the convulsive action of insulin than are normal rats. It might be argued, therefore, that removal of the pituitary results in a relative excess of insulin in the body and that the greater proportion of carbohydrate used for oxidation is due to this factor. Houssay has shown, however, that an extra-pancreatic factor is also involved, since extirpation of the pituitary in depancreatized dogs enabled them to oxidize carbohydrate.

Summary. Hypophysectomized rats continue to oxidize carbohydrate for a longer period of time during fasting and they derive a larger proportion of their energy from carbohydrate after glucose feeding than do normal rats. A high fat diet prior to glucose feeding reduces carbohydrate oxidation in normal rats but has little effect in hypophysectomized rats; the latter oxidize more sugar in the first 4 hours after the glucose feeding than the former in spite of a lower O_2 consumption and a reduced rate of intestinal absorption.

8519 C

Effect of Acid Extract of Cattle Ant. Pituitary on Bone Repair in Thyroidectomized Guinea Pigs.

MARTIN SILBERBERG AND RUTH SILBERBERG. (Introduced by L. Loeb.)

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In former investigations we have shown that the growth-promoting influence of acid extract of anterior pituitary gland of cattle on bone and cartilage of young guinea pigs is exerted without the intervention of the thyroid gland.[1] Correspondingly we wished to determine whether the accelerated healing tendency of fractured bones, caused by the extract,[2] is also independent of the action of the thyroid.

The experiments were carried out in 26 winter and spring guinea pigs, on the average weighing from 120 to 280 gm. Under deep ether anesthesia the tibia and fibula of the right hind leg were broken about the middle of their shafts and bandaged according to the method described previously. Both lobes of the thyroid were completely removed immediately after fracturing. Injury to the parathyroids was carefully avoided. Sixteen of the animals were injected daily with 1 to 1½ cc. of extract intraperitoneally for periods varying from 6 to 21 days, the first injection being applied immediately after thyroidectomy. Ten guinea pigs were not injected and served as controls. After 6, 10, 14, and 21 days the fractured bones were removed *in toto* and specimens prepared for microscopical examination as previously described.[3]

As to the gross appearance of the callus in the injected and thyroidectomized animals, it was invariably firmer and harder than that of the non-injected thyroidectomized guinea pigs. This fact was noted as early as 6 days after fracturing, but even more distinctly at subsequent periods. In all cases a complete union by a dense, bony, calcified callus had taken place after 21 days, while in the non-injected animals the callus was looser and softer at that period. There was no marked difference in gross appearance between the callus in injected thyroidectomized guinea pigs and in those with intact thyroid glands, which had been injected.

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[1] Silberberg, M., PROC. SOC. EXP. BIOL. AND MED., 1936, **33**, 554.

[2] Silberberg, M., and Silberberg, R., PROC. SOC. EXP. BIOL. AND MED., 1935, **33**, 177.

[3] Silberberg, M., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1423.

Microscopical Examination. First Series. In thyroidectomized guinea pigs as early as 6 to 7 days after fracturing, the ends of the broken bones have become calcified. They are separated by a newly-formed vascularized granulation tissue representing a loose connective tissue callus. In addition a reaction of the periosteum has taken place, though it is considerably delayed in comparison with that of the normal animals.[2] In some areas the very beginning of a conversion of the mesenchymal cells into osteocytes and an osteoid tissue is seen. But, on the whole, this new formation of osteoid tissue is inconsiderable. Neither cartilage formation nor any calcification of the osteoid tissue can be observed.

After 10 days a union of the ends of the broken bones by osteoid tissue has occurred, but a strong growth tendency is missing, as indicated by a diminished formation of cartilage and bone and by the very small number of mitotic figures which are seen.

Fourteen days after fracturing a small but definite callus is found; it is apparently less developed than under normal conditions, and the formation of osseous tissue and particularly that of the cartilage is very limited. The vesicular cartilage cells show dense nuclei and a very small amount of light cytoplasm. In comparison with the healing process in normal animals not only is the development of the cartilage considerably diminished but also its calcification is retarded.

Likewise, after 21 days the healing tendency is still very slight, as demonstrated by delayed and by decreased new formation of cartilage, while the osteoid substance is better developed, but evidently less so than normally. In addition, the calcification of the vesicular cartilage is retarded and little osteoclasis is present.

Second Series. In thyroidectomized animals injected daily with extracts of anterior pituitary gland, after 6 to 7 injections a definite union of the broken stumps by osteoid tissue, originating from the periosteum, is seen; the dense callus which thus develops is of considerable size. Large islands of vesicular cartilage distinctly basophilic and tending to become calcified are found. These changes are even somewhat more pronounced than in injected non-thyroidectomized guinea pigs. The cartilage cells show hypertrophic cytoplasm and large nuclei as well as an increased number of mitotic figures. The cartilage appears chiefly in areas that are surrounded by osteoid tissue, in which latter likewise a stimulation of growth processes is noticeable.

As early as after 10 injections the callus is already calcified and ossified, the markedly basophilic cartilage cells exhibiting at this time extreme hypertrophy and hyperplasia.

After 14 injections a considerable amount of osseous and cartilaginous callus is present, together with hypertrophy and hyperplasia of its cells. At that date the process of calcification has further progressed than in the thyroidectomized controls after 21 injections. In addition, a good many mitotic figures and osteoclasts are noticeable. The difference between the injected and non-injected thyroidectomized guinea pigs is very marked. In some cases of this series the process of bone repair seems to be even more accelerated than in the guinea pigs with intact thyroid under the influence of the extract.

After 21 injections there has developed a hard, dense, bony callus connecting the broken fragments. Osteoclasis as well as vascular absorption go hand in hand with the formative processes as described above, thus demonstrating a well-balanced healing tendency.

In contradistinction to the behavior of the callus in injected guinea pigs with intact thyroids, where in about 20% of the cases acceleration of calcification was absent, in the above experiments a uniform acceleration was obtained. The assumption seems, therefore, to be justified that some conditions inherent in the thyroid gland are responsible for the differences between these 2 classes of animals.

Conclusions. In thyroidectomized controls, callus formation is retarded as compared with the findings in normal guinea pigs. In particular, the production of cartilage is delayed and diminished. Osteoid tissue is also less developed. Under the influence of the extract, however, a faster transformation of mesenchymal tissue takes place (1) directly into osteoblasts, osteoid tissue and bone; (2) indirectly into cartilage and afterwards into bone by the way of endochondral ossification.

Thus anterior pituitary extract of cattle counteracts and overcomes the growth-preventing influence on cartilage and bone of young guinea pigs exerted by thyroidectomy and causes an accelerated healing tendency of the fracture and a faster consolidation of the callus. We may, therefore, conclude that the extract exerts its influence independently of the thyroid gland. There is under these conditions a parallelism between the process of endochondral ossification in the epiphyseal line and the healing of the fractures by means of a callus.

8520 P

Inhibition of Brown-Pearce Rabbit Tumor with Filtered Homologous Tumor Material.

ALBERT E. CASEY.

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In the course of some experiments with the Brown-Pearce rabbit tumor[1, 2, 3] an unusual inhibitory effect was noted following a single injection of 0.3 cc. of a Berkefeld "V" filtrate of fresh tumor given 2 weeks before the tumor inoculation. The experiment had not previously been tried and has not yet been repeated. It would not be reported here except that it is the only case of complete failure of the Brown-Pearce rabbit tumor to grow following intratesticular inoculation in some 7 years of experience involving approximately 100 different transplantations of the tumor, and approximately 1000 rabbits. This experience includes studies on some 20 pure breeds, and on various types of hybrids, particularly with the type of brown-gray hybrids used in the experiment; and the probability of a rabbit being free of tumor at the end of a 2 months' observation period was calculated to be 0.2306. The occurrence of all 6 animals in an experiment being negative by accident is, therefore, very unlikely ($P = 0.01$—).

Furthermore the 6 animals of this unusual group were identical as to source, age, weight, coat color, vigor, size of testicles, and body build with 36 other rabbits not receiving the filtrate and composing 6 additional groups inoculated on the same day in staggered order[3] in the same testicle with the same dosage of the same fresh tumor. One of the 6 additional groups was a control group and was not otherwise treated. Analyzed per animal inoculated, the incidence of primary tumors in this group was 50% and the tumors averaged 5.8 cc.; the incidence of metastatic tumors was 83.3%; the metastatic tumor averaged 53.0 cc. in volume per animal and was distributed over 6.3 metastatic foci; the mortality was 61.1% over a period of 59 days. The 5 additional groups were treated with various other homologous materials before tumor inoculation and had an incidence of primary tumors of 40% which averaged 3.8 cc.; the inci-

* The work was aided by a grant from the International Cancer Research Foundation.

1 Casey, A. E., *Am. J. Cancer*, 1934, **21**, 760.

2 Casey, A. E., *Am. J. Cancer*, 1934, **16**, 776.

3 Casey, A. E., *Am. J. Cancer*, 1936, **26**.

dence of metastatic tumors varied from 80 to 100% per group, and averaged 88.0%; the volume of metastatic tumor per group varied from 40 to 162 cc. per animal and averaged 94 cc.; the number of metastatic foci varied from 10.2 to 24.2 per group and averaged 13.9 foci; the mortality varied from 60 to 100% and averaged 71.4%; the longevity varied from 48 to 61 days and averaged 54.7 days. In contrast with this only one of the 6 animals receiving the filtrate of fresh tumor 2 weeks before the tumor inoculation developed a primary tumor and this was a small, entirely necrotic nodule of 3 cc. at the end of the experiment, an average per animal inoculated of 0.5 cc.; the incidence, volume and number of metastatic foci were 0 for the group; the mortality 0, and the longevity 61 days (termination of the experiment). This result was statistically at variance with the results in the other groups inoculated. This inhibition could not be explained on the basis of the materials and methods. None of the animals were of resistant strains such as, the Havana, Polish, and Himalayan breeds, and so far as could be determined were identical with the animals of the other groups.

No other explanation is at present tenable other than that the filtrate of the fresh Brown-Pearce tumor contained a material exerting an inhibitory effect on tumor transplantation. This cell-free filtrate suggests the inhibitory material found in chicken tumors and normal tissues of various animals by Murphy, Helmer, Claude, Sturm,[4] and others. The method of testing differs, however, in that the present filtrate was injected in a different site 2 weeks before the tumor inoculation, instead of being mixed with the tumor inoculum. The source differs in that it was obtained from and exerted its effect upon the same mammalian tumor. Attempts to confirm this observation will be made and the success or failure reported.

[4] Murphy, J. B., Helmer, O. M., Claude, A., and Sturm, E., *Science*, 1931, **73**, 268.